THE THESIS
ON

A STUDY OF THE NON-TRADITIONAL OILS AND THEIR FATTY ACIDS

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BY
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CERTIFICATE

Certified that the work contained in the thesis entitled " A STUDY OF THE NON-TRADITIONAL OILS AND THEIR FATTY ACIDS" has been carried out by Mr. Yousuf Akhtar Roomi, under my supervision and the same has not been submitted elsewhere for a degree

(S.Q. Hasan)
Supervisor

ACKNOWLEDGEMENTS

I am deeply indebted to my supervisor, Dr. Syed Qamarul Hasan, for his valuable guidance, esteemed inspiration, stimulus and constructive suggestions at every stage of my work. It is his whole hearted support , cooperation and meticulous scientific attitude which inspired me and also enabled me in accomplishing this venture.

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My family members have contributed a lot in the formation of this thesis. It is their encouragement as well as support which has given me a lot of enthusiasm at every stage of my research work. This thesis is a result of their blessings and good wishes. I really owe each of them.

I feel immense pleasure and satisfaction in dedicating this thesis to my father Janab Abdul Mateen Roomi , who has all along been a source of inspiration and guidance towards the RIGHT PATH.

YOUSUF AKHTAR ROOMI

CONTENTS

SUMMARY

1. SUMMARY

The work presented in this thesis consists of two parts.

Part I concerns with the compositional studies on indigenous seed oils.

Part II deals with the reactions of Long-chain internal, terminal and α,β-unsaturated acids and their derivatives with nitrosyl bromide (NOBr).

PART I

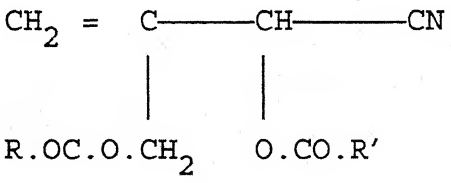
Compositional Studies on Indigenous Seed Oils

1. Non-glyceride component of oils: Cyanolipids

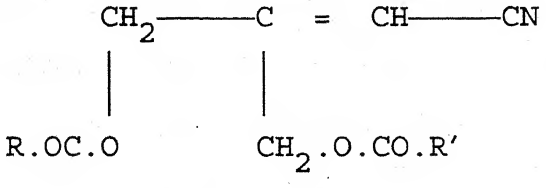
Cyanolipids are a new class of plant lipids which found often in copious amounts in seed oil of some members of sapindaceae family. Previously four types of cyanolipids present individually or in pairs have been reported in the seed lipids of sapindaceae and boraginaceae species.

In the present investigation three sapindaceous seed oils namely *Sapindus saponaria, S. trifoliatus* and

Lepisanthes tetraphylla along with four seed oils from boraginaceae family namely *Heliotropium indicum, Heliotropium eichwaldi, Cordia obliqua (C. wallichii)* and *Cordia dichotoma* were investigated for their cyanolipid contents. During compositional studies on indigenous seed oils we have isolated and characterized this new class of plant lipid in *S. saponaria, S. trifoliatus* and *L. tetraphylla* seed oils. *L. tetraphylla* seed oil was found to contain a fatty acid diester of 1-cyano-2-hydroxymethylprop -2-ene-1-ol (I, 22% w/w). However *S. saponaria* and *S. trifoliatus* seed oils were found to contain a fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol in varying proportions (II, 15, 20% w/w) and their structures were corroborated on the basis of spectroscopic and



I



II

The present work on *Heliotropium indicum, H. eichwaldi, Cordia obliqua, C. dichotoma* of boraginaceae family revealed the absence of cyanolipid in all these four species. Thus the distribution of cyanolipid seems to be limited to member of sapindaceae family only.

2. Fatty acid analysis of indigenous seed oils

The seed oils from seven species of sapindaceae and boraginaceae family, were analyzed for their component acids mainly by chromatographic and spectroscopic techniques. These seven seed oils were found to contain usual type of oleic-linoleic-linolenic acids but in varying proportions. Gas-liquid-chromatographic (GLC) analysis confirms that no significant amount of unusual fatty acids were present, and they are composed of conventional fatty acids but in varying proportions. The total content of saturated acids ranged from 8.3-38.2%. In the combined content of palmitic and stearic acid, palmitic was found to be present as a major component in all the seven samples, which is the usual pattern of distribution of palmitic and stearic acid. Other than C_{16} and C_{18} saturated acids C_{20} and C_{22} saturated acids were also found to be present in various species in minor amount. C_{22} saturated acid (Behenic) was found to be occurring in three species namely,

S. saponaria, L. tetraphylla and *C. dichotoma*, to the extent of 1.2-3.5%.

The total of unsaturated acids in the species examined varied from 66.8-91.7% in the triglyceride fractions. C_{18}-unsaturated acid ranged from 50.4-89.9%. Among C_{18}-unsaturated acids oleic and linoleic acids were found to be the most frequently occurring acids rather than linolenic acid which was present as minor constituent ranging from trace to 06.1%. The combined content of oleic and linoleic acid was found to vary in the region 50.4-83.8%. A moderately high percentage 21.4% of C_{20} monoenoic (eicos-11-enoic) was found to be occurring in L. *tetraphylla*. Two species namely *H. indicum* and *and H. eichwaldi* yielded moderately linoleic-rich seed oils containing 34.1-44.1% of linoleic acid. The triglyceride fractions and nitrogen-containing-lipid-fraction (NCLF) of all the three sapindaceons seed oils were found to be rich in oleic acid ranging from 48.6-62.6%. The eicosanoic acid was found to be present in substantial amount in both the fractions (TG and NCLF) of sapindaceous seed oils ranging from 12.3-30.8%. The present work also revealed the preferential incorporation of saturated C_{20} acids in NCLF.

Oil from *H. eichwaldi* contained 44.1% of linoleic acid with no linolenic acid may be grouped as semi drying oil, whereas, *S. saponaria, S. trifoliatus, L. tetraphylla, H. indicum, C. obliqua (C. wallichii)* and *C. dichotoma* contained less of linoleic acid and no or very small amount of linolenic acid belong to the group of non-drying oils. A serious consideration can be given to species rich in oils as well as in specific acids if any can meet the agronomic standards of a field crop.

PART II

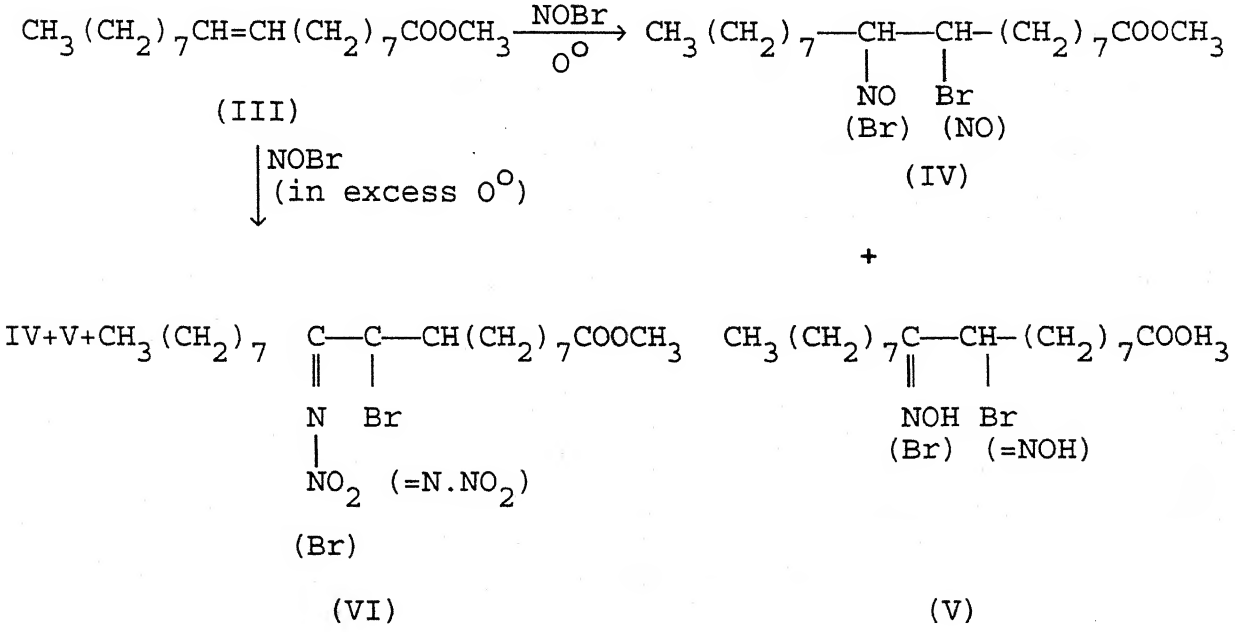
In a continuing study on the reaction of long-chain fatty acids, an attempt was made to study the action of nitrosyl bromide upon internal, terminal, and α,β-unsaturated acids and their derivatives.

A. Nitrosobromination of methyl oleate

The nitrosobromination of methyl oleate with approximately stoichiometric quantities of NOBr *in situ* yielded chiefly nitrosobromo product (IV,methyl 9(10)-bromo-10(9)-nitrosooctadecanoate), accompanied with some amount of its isomeric oximino form (V, methyl 9(10)-bromo-10(9)-oximinooctadecanoate). The excess of NOBr

yielded an unusual product bromonitrimine (VI, methyl 9(10)-bromo-10(9)-nitriminooctadecanoate) in addition to normal products (IV and V, Chart I). The formation of bromonitrimine can well be explained through the oxidation of oxime (V). The structure of products were established by microanalysis, IR and NMR.

Chart I

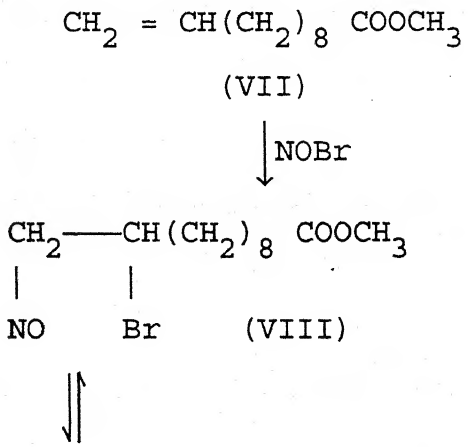


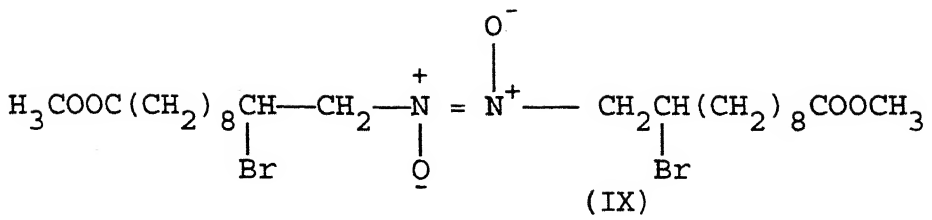
B. Nitrosobromination of methyl 10-undecenoate

In the present work the methyl ester of 10-undecenoic acid (VII) was selected as a model substrate for nitrosobromination reaction for two reasons. Firstly,

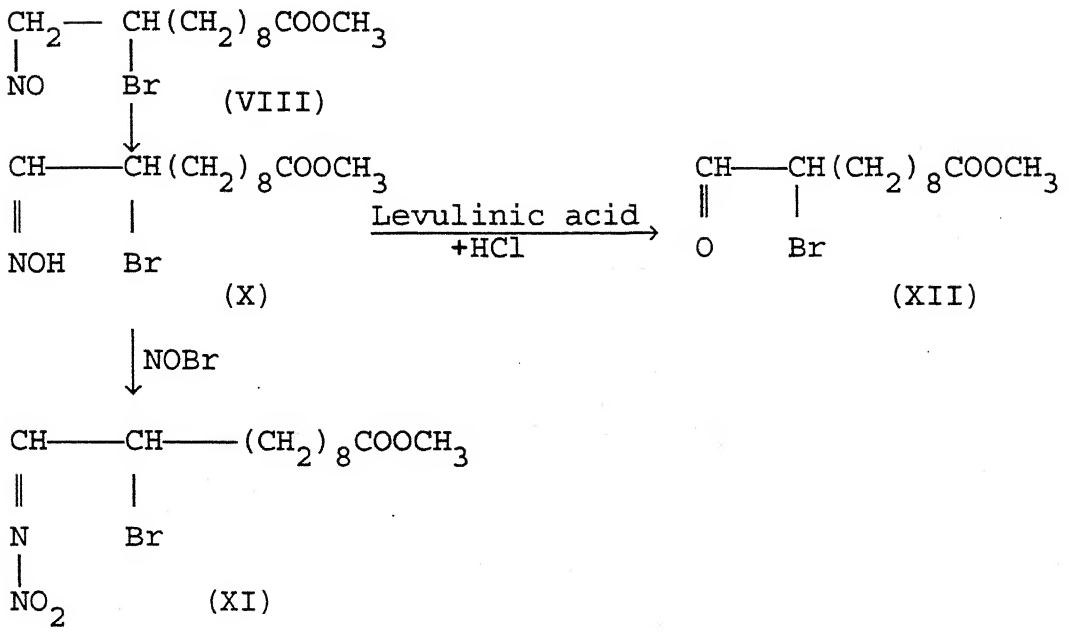
there appeared to be no mention in the literature of the nitrosobromination of undecanoic acid. Secondly, it was considered desirable to probe the regioselectivity of nitrosyl bromide addition on an unsymmetrically substituted double bond as well as the stearic effect in the formation of product. Reaction of methyl 10-undecenoate (VII) with NOBr *in situ*, resulted in the formation of four products (VIII-XI, Chart II). Only three components (IX–XI) could be isolated and characterised in pure form viz. dimer of methyl 10-bromo-11-nitrosoundecanoate (IX), methyl 10-bromo-11-oximinoundecanoate (X), and methyl 10-bromo-11-nitrimino-undecanoate (XI). The compound (VIII, methyl 10-bromo-11-nitrosoundecanoate), a primary product, could not be isolated in pure form as it easily dimerizes or rearranges to an oxime.

Chart II





(IX)



(VIII)

(X)

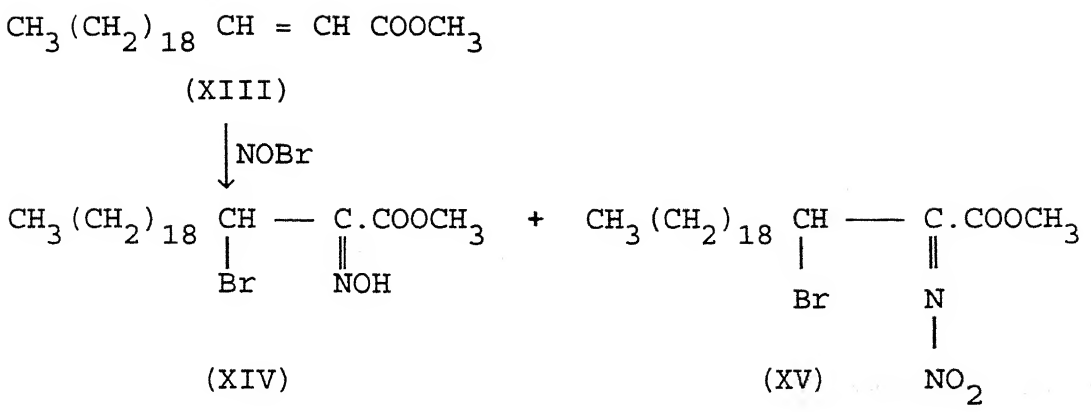
(XII)

(XI)

C. Nitrosobromination of docos-*trans*-2-enoate (XIII)

When methyl docos-*trans*-2-enoate was treated with NOBr (*in situ*) at 0.5°C for about a month only about 10% of the compound (XIII) was found to undergo nitrosobromination. The products (XIV and XV, Chart III) were characterized as methyl 2-oximino-3-bromodocosanoate respectively on the basis of elemental analysis, IR and NMR.

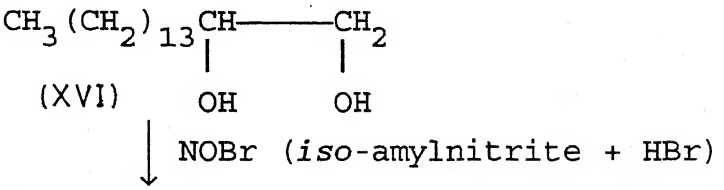
Chart III

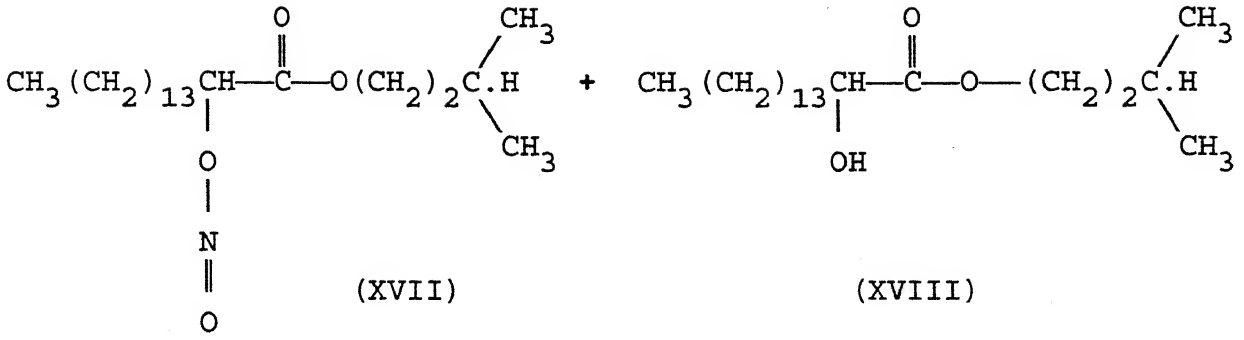


D. Reaction of nitrosylbromide with fatty acid 1,2-diol (1,2-hexadecandiol, XVI)

The reaction of the vicinal diol (XVI) was carried out with nitrosyl bromide (NOBr, *in situ*) in alcohol at room temperature. The reaction yielded *iso*-amyl 2-nitritohexadecanoate (XVII) and *iso*-amyl 2-hydroxy hexadecanoate (XVIII). The products were identified on the basis of microanalysis, IR, NMR and Mass. The reaction is depicted in Chart IV.

Chart IV



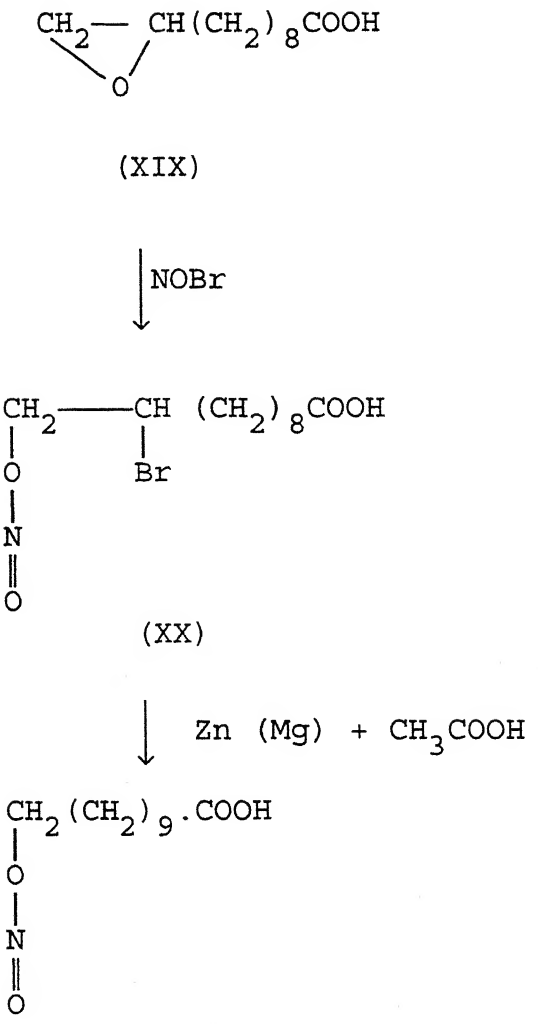


(XVII) (XVIII)

E. Reaction of nitrosylbromide with 10,11-epoxyundecanoic acid (XIX)

The present work on NOBr rection with epoxide (XIX) was undertaken primarily as there appeared to be no mention of NOBr with oxiranes. Further a terminal epoxide was selected as a subsrate to study the direction of ring opening. When 10,11-epoxyundecanoic was treated with NOBr a quantitative yield of 11-nitrito-10-bromoundecanoic acid (XX) was obtained and reaction is depicted in Chart V.

Chart V


CH2— CH(CH2)8COOH
O
(XIX)
NOBr
CH2——CH (CH2)8COOH
O Br
N
O
(XX)
Zn (Mg) + CH3COOH
CH2(CH2)9.COOH
O
N
O

INTRODUCTION

INTRODUCTION

Although the study of natural products has always been a prominent part of organic chemistry, fatty acids have not been seriously considered in comparison with the more favoured carbohydrates, alkaloids and isoprenoids to mention only a few. Fatty acids generally undergo all chemical reactions of organic chemistry. It is important to note that the various phases of the development and progress of organic chemistry are better exemplified by the general perfection achieved by the Lipid chemistry.

The long-chain fatty acids have their origin in land and marine animal fats, vegetable seed oils and organic synthesis. Naturally derived fatty acids are normally considered replenishable in the sense that animal and plants reproduce themselves. There are good reason for expecting that fatty acids as renewable sources, will become even more important in years to come. There has been a concentration of a study on only two acids oleic and linoleic and their geometric isomers. It has generally been assumed that what is true of oleic acid hold for other monoenoic acids and that what is true for linoleic acid holds for other polyenoic acids. Considering this limited arena of investigation in fatty acid chemistry our research activity on fatty acid reactions has been directed towards the

reactions of olefinic acids containing terminal, internal, and α-β-unsaturation. Our emphasis has been more on the preparation of new fatty acid derivatives using both classical and nonclassical reactions and to study the co-relation of structure and product composition in the preparation of these derivatives.

It has been estimated that by the turn of the present century the world population will be doubled and a serious global crisis will arise for the need of oil in the edible and non-edible industries. Consequently the programme of screening uncultivated seed oils has been initiated in many advanced countries with a view to discover new seed oils and fatty acids which may lend themselves to practical utilization. In sixties India used to export large quantities of oil seed after meeting its domestic requirements. The situation has, however, changed in the past due to rapid population growth and the country has now become a major importer of oils and fats. There is a vast potential of minor oil seeds which if properly trapped, can substantially augment the overall supplies of vegetable oils and help in bridging the wide gap between their demand and supply. The growing demand of fatty chemicals as raw materials for use in oil-based industries has in recent years diverted the attention of Lipid chemists from the analytical aspect of fats to the chemistry of fatty acids. Uncertainty over availability and cost of petrochemicals has

rekindled academic interest in natural oils as an alternative raw material source of fatty chemicals. In view of aforesaid objectives the present work described in the thesis deals with the studies on seed oils and chemistry of fatty acids.

PART -I

COMPOSITIONAL STUDIES ON INDIGENOUS SEED OILS

A. THEORETICAL

Of the various sources of oils and fats, edible vegetable oils constitute half of the world production of oils and fats. In last two decades the world-wide production and consumption of fats and oils has gone up by approximately two million tonnes, or some 2.5% a year. The greatest increase in production has been recorded for soyabean, palm, rapeseed and sunflower seed oil. The share of developing countries in the growth has been comparatively small, although some of them such as Malaysia, Brazil, Indonesia and the Ivory cost, have increased the production and export tonnage in recent years.

Our knowledge of natural fats of vegetable origin is very limited to the extent that out of about 2,50,000 species of plants, seed oils from about 12-13,000 plants have so far been screened and subjected to fatty acid analysis. Lipid chemists have been actively engaged in basic and applied research aimed at the development of new crops for use in oil-based industries and edible purposes. The fundamental phase of this type of research is a cooperative screening programme to discover, define and evaluate new or unusual compounds of promising utility in many different directions in plants with a reasonable

potential for cultivation. Such screening programme has revealed and continues to indicate seed species whose development into new domestic crop could satisfy existing needs, or newly developing requirements of our oil-based industry as it increases in size and complexity. The species or groups of species, found to have an outstanding potential as new oilseeds, await agronomic improvement through selection and breeding before crop status can be realized.

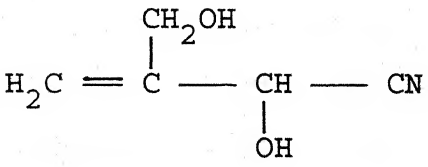
India has vast unexploited resources of wild herbaceous flora. Thus there is a need for a phytochemical screening of oil-bearing plant species, to develop better substitute for conventional oils and oleochemicals.

In past years, lipids were considered to be oily intractable substances that could be separated into simpler components only with great difficulty and they were studied by a comparatively limited number of painstaking researchers. The development of chromatographic techniques, particularly gas-liquid chromatography and thin-layer chromatography, together with advances in spectroscopy, have led to an explosive growth of interest in these compounds and have revolutionized our knowledge of the role that lipids play in the structure and function of cell membranes, as essential dietary components and in numerous biological processes.

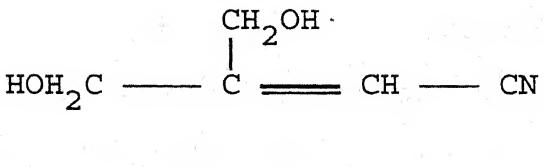
Keeping in view the line of work described in this thesis an attempt will now be made here to mention briefly the non-glyceride and non-glyceride components of oil-bearing seeds and analysis of their fatty acids.

Non-Glyceride Components of Oils : Cyanolipids

Cyanolipids are a new class of plant lipids which are found, often in copious amounts, only in the seed oils of sapindaceae plants and probably play an important role in biochemistry of these plants. Cyanolipids are not glycerides but they are derivatives of five-carbon mono or dihydroxynitrile moiety (I-IV) esterified with long chain fatty acids. Although the occurrence of cyanolipid materials has been suspected for many years[1,2], due primarily to the cyanogenic property of *Schleichera trijuga*[3] (commonly known as kusum) seed oil of sapindaceae family. In early 1970's their structures have been determined and the existence of cyanolipids acknowledged[8,9].



(I)



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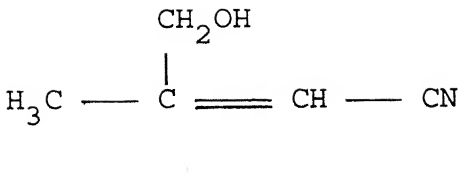

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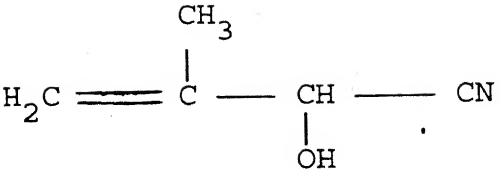


(IV)

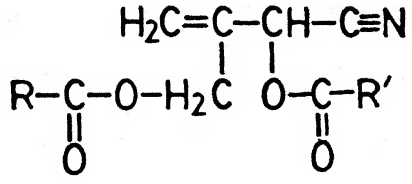
The unifying structural feature of these cyanolipids is that they are based on the same branched, five-carbon nitrile skeleton, although the double bond position and the number and location of hydroxyl groups are not the same. Two of the four known cyanolipids do not fit in the "diol lipid" category[10] because they are monoesters, and cyanolipids as a group can not be labelled "Cyanogenic Lipids" because two of them do not liberate HCN, (II,III). The unique structures of this new class of lipids enable them to undergo reactions (as well as to be derived from biogenetic pathways) that are distinctive from other lipid class.

Progress in cyanolipid identification began with reports[11-14] on *Schleichera trijuga* seed oil, even though their data and conclusions were quite misleading. At about the same time Mikolajczak *et al.*, in series of publication[3-7,15] described the detection isolation and structure proof of four types of cyanolipids having different but closely related structures. In 1920, cyanolipids were first observed in *Schleichera trijuga*

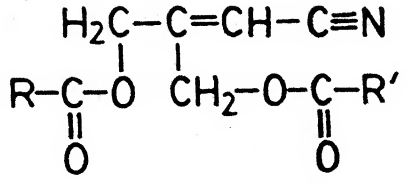
(sapindaceae) seed oil by Rosenthaler[1] and SenGupta[2]. But the exact location of the cyanogenic moiety in the oil or its exact nature was not reported. The compound has been suspected to be in the form of a cyanogenic glucoside or an acid amide[16]. Later, Kundu *et al.*[14] reinvestigated the same seed oil to ascertain the location and nature of the cyanogenic compound by applying chemical methods, chromatography and infrared spectroscopy. Observations indicated the cyanogenic compounds to be a part of glyceride molecule in which one of the hydroxyl group of the latter is bonded to the cyanogenic compound through an ether linkage. Chromatographic behaviour of the isolated cyanogenic compounds further indicated that at least two glyceride molecules are involved. These glycerirdes are predominantly esterified with saturated fatty acids. At the same time Kasbekar and Bringi[11] working on the same seed oil found with the help of TLC that the oil is composed of approximately 37% of glyceride, the rest being non-glycerol esters of fatty acids.

Later studies[3-7,15,17-19] have shown that the cyanogenic material is non-glycerol ester composed of one or two ordinary fatty acid moieties (predominantly C_{-20}) esterified with an unsaturated isoprenoid hydroxy or dihydroxynitrile.

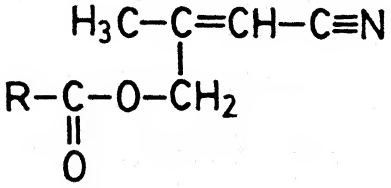
Four types of cyanolipids (V-VIII), present individually or in pairs, have been identified in the seed lipids which are cyanogenetic nonglycerol esters and are derivatives of five-carbon mono or dihydroxynitrile moiety esterified with long chain fatty acids (V - VIII). Out of these, one class of compound is a mixture of diesters containing two fatty acids moieties esterified with 1-cyano-2-hydroxymethylprop-2-ene-1-ol (V) and 1-cyano-2-hydroxymethylprop-1-ene-3-ol (VI). The other class of cyanolipids comprise mono-ester of one cyano-2-methylprop-1-ene-3-ol (VII) and 1-cyano-2-methyl prop-2-ene-1-ol (VIII).



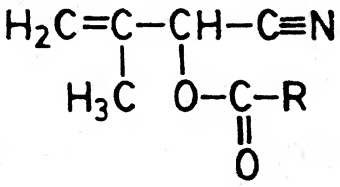
[V]



[VI]



[VII]



[VIII]

Each cyanolipid fraction is a mixture in which the constituents differ only in the attached fatty acids; because this mixture was difficult to separate and appeared to be based on a single aglycone, it was treated as a single

entity during the course of investigation.

Significantly, the hydroxynitrile moiety in all four cyanolipids has an isoprenoid skeleton this permits numerous possibilities for its biosynthesis. Since other natural cyano compounds often seem to be derived from amino acids or their precursors[20-22], it should be noted that decarboxylation of L-leucine would give the requisite saturated carbon and nitrogen skeleton for these nitriles.

A curious feature of these cyanolipid - containing seed oils is their high percentage of C_{-20} acids and the preferential incorporation of these acids into cyanolipids rather than into the accompanying triglycerides. This preference probably related to the observation that *Litchi chinensis* seed oil, which contains insignificant amount of C_{-20} acids, also devoid of cyanolipids[15]. Additional sapindaceae seed oils that are low in C_{-20} acid should be examined for confirmation of this view.

More recently the presence of cyanolipids has been shown in *sapindus saponaria, sapindus trifoliatus* and *Lepisanthes tetraphylla* of sapindaceae family by author.

Cyanolipids have thus far been found only in seed oils of sapindaceae plants, however, not all sapindaceous seed oils contain cyanolipids[3-7,15]. Even though, oils from plants of other families have been screened for cyanolipids,

but none have been detected. *Schleichera trijuga* seed oil yields the largest total amount of cyanolipid, but seed oils of some *Koelreuteria, Cardiospernum* and *Paullinia*[1,3,15] species are close behind. Of course, seeds from different accessions may display differing amounts of cyanolipids depending on such variables as growing location, seed maturity and storage conditions. Various seed oil of sapindaceous plants have been analyzed for cyanolipid contents by Seigler[123] but no new cyanolipids were found. A comparative study of cyanolipids revealed that the cyanolipid (V) is by far the most abundant and usually occurs either or with a minor amount of cyanolipid (VI), while cyanolipid (VII) appears, for the most part, in combination with cyanolipid (VI). Whereas no oil, examined to date has contained all four cyanolipids. Cyanolipid (VIII) has been detected in only one seed oil, that of *Ungnadia speciosa*[15,18]. A review of the literature reveals that members of a particular genus usually produce the same cyanolipid(s).

Both the column chromatographic and preparative TLC proedures[15] used for the isolation of cyanolipids, which are somewhat unstable especially on hydrolysis, are time consuming; therefore Seigler[18] has developed the use of NMR spectra of the chloroform extracted seed oils to determine the presence and amount of cyanolipids and the glycerides with which they occur. All four cyanolipids (V-VIII) may be

distinguished from each other and from glycerides by this method.

The search for cyanolipids in plants other than sapindaceae has been extended by Seigler and Kawahara[123] to include members of four families viz. Meliosmaceae, Melianthaceae, Hippocastanaceae and Staphyleaceare which are closely allied botanically to the sapindaceae, but none of seed oils examined gave any indication of the presence of cyanolipids.

Component fatty acids of natural fats

Hitherto more than 600 fatty acid structures have been reported and the following generalization can be drawn from these. Usually fatty acids contain an even number of carbon atoms and are most commonly C_{16}, C_{18}, C_{20} or C_{22} compounds : Unsaturated acids have double bonds with *cis* or Z configuration in certain preferred positions in the carbon chain : polyene acids have have methylene - interrupted unsaturation; the acids rarely contain any functional group other than the carboxyl group and olefinic unsaturation. Of course there are exceptions to all these statements. Nevertheless, there are valuable generalizations against which to discuss fatty acid structure. For last three decades, many new fatty acids possessing structural features quite unusual according to our earlier concept, have been

discovered at an unprecedented rate.

The structural studies of these unusual fatty acids of plant origin have been comprehensively reviewed by Smith[25]. The unusual structural features of recently discovered natural fatty acids are described in subsequent headings.

Fatty acids containing unusual unsaturation

In recent years among C_{18} acids, mono-unsaturation was found at different positions, i.e. 3,5,6 and 11. All *cis*-5,9 and 5,9,12 acids have recently been reported in the seed oils of *Taxus baccata*[26] and *Laryx leptolepia*[27] respectively. The ω-5 monoenes have been found in the seed fat of *Grevillea robusta*[28]. C_{16} mono-saturation in seed fat is not as common as C_{18} mono-unsaturation, though amongst these acids mono-unsaturation has been found at the position, 3,5,6,7 and 9. Spencer *et al.*[29] have reported the presence of 82% hexadec-*cis*-6-enoic acid in *Thunbergia alata* seed oil. Later Osman *et al.*[30] have reported that the presence of 15.4% hexadec-*cis*-9-enoic acid in *Zanthozylum alatum* (Rutaceae) seed oil.

Until 1964 only one acetylenic acid, tariric acid i.e. octadec-6-ynoic acid was known. Since then a number of acetylenic fatty acids have been discovered in the oils of plant families Olacaceae, Compositae, Santalaceae and

Simaroubaceae. Polyunsaturated acetylenic acids have been found in plant seeds from only two families, Olacaceae and Santalaceae. Ligthelm and Schwartz[31] proposed four possible structures for an unknown acetylenic acid in seed oil of *Ximenia caffra*, and proposed it be named Ximenynic acid. Latter, Ligthelm et.al. characterized the acid as octadec-trans-11-ene-9-ynoic acid[32]. Pearl et.al.[33] reported two previously unknown acetylenic acids in the seed oil of *Alvaradoa amorphoides* of simaroubaceae family. These acetylenic acids were characterized as octadec-17-ene-6-vynoic and eicos-6-ynoic acids.

A number of seed oils of Labiatae family have been analyzed by several workers[34-36] and found that the subfamily stachyoidae is unique in the frequency with which allenic acid occurs. The first allenic acid was reported in Labiatae family by Wolff *et al.*[34] to be (-) octadeca-5,6-dienoic acid (Laballenic acid). Osman *et al.*[37] have reported that the seed oil of *Leucas cephalotus* (labiatae) to be the richest source of (-) octadeca-5,6-dienoic acid (Laballenic acid).

Oxygenated Fatty acids

Epoxy acids

Epoxy fatty acids are well distributed in seed oils of various plant families, out of which four viz., Compositae, Dipsacaceae, Euphorbiaceae and Malvaceae, may be

considered of major importance, since they incorporate large number of species with seed oils more or less rich in epoxy acids[38]. Not counting enantiomeric forms and excluding some additional epoxy acids in cutins, one C_{20} and nine C_{18} *cis*-epoxy acids have been isolated from seed lipids[39-45]. However they may be formed in nature and this remains an unsolved problem, they can be considered as oxidation products of olefinic acids. The structure can be arranged to show their relationship with the appropriate alkene acid: Oleic acid in the first group, linoleic and crepenynic in the second group and linolenic and other triene acids in the final group. There are five 9,10-epoxides three 12,13-epoxides and one 15-16-epoxide. Vernolic was the first acid of this class to be discovered by Gunstone in 1954[43], and this acid was structurally characterized as *cis*-12,13-epoxy-*cis*-9-octadecenoic acid and it is still the most readily available.

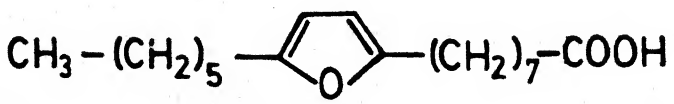
A C_{20} homologue (alchornaic acid) of vernolic acid has been discovered by Kleiman *et al.*[44] from the seed oil of *Alchornea cordifolia* of Euphorbiaceae family. C_{18}-epoxy acids were reported in the seed oil of *Crepis conyzaefolia* (Goun), Dalle Torre (compositae) by Spencer[45],are (+)-*cis*-12,13-epoxyoctadeca-*trans*-6-*cis*-9-dienoic (1 %) and *cis*-12,13-epoxyoctadeca-*cis*-6-*cis*-9-dienoic (2%) acid. A new 3,4-epoxy acid, *cis*-3-4-epoxyoctadec-*cis*-11-enoic (17. %) acid was reported in the *V. roburghii* seed oil[46]. Other

C_{18}-epoxy acids were reported in the seed oil of *Mucuna prurita* (Legumnosae) by Hasan and coworkers[47], are *cis*-12,13-epoxyoctadec-*cis*-9-enoic and *cis*-12,13-epoxyocadec-trans-9-enoic acid.

Furanoid Fatty Acids

In the group of natural ether acids which contain a furan ring the first represents an unbranched 18-carbon furanoid acid with an ether linkage between C-9 and C-12 or, alternatively the C-10 and C-7. This acid (9,10-epoxyoctadeca-9,11-dienoic, IX) was first isolated in 1966 by Morris *et al.*[48] from seed oil of *Exocarpus cupressiformis* (Santalaceae). It remained a chemical curiosity until the report in 1975 by Glass *et al.*[49-51] that the lipids of the male Northern Pike (*Esox Glucicus*) contain six or more branched-chain furanoid acids.

We know little or nothing of the biosynthesis, metabolism, or purpose of these unusual acids. There is growing interest in the oxygenated acids which appear in seed oils after prolonged storage of the seeds. The



[IX]

occurrance of such acids in commercial seed oils makes it important that we know more about their chemistry. Sunflower seed oil, extracted from seeds which have been stored for 2-10 years, contain about 5% of oxygenated acids among which four have been recognized: they include 9,10-epoxy acids related to oleic and linoleic acid and two of the hydroxy diene acids which arise, presumbly, through oxidation of linoleic acid[52]. The seed oil of *Stenachaenium macrocephalum* is unusual in containing a rare triene acid with 3*t* 9*c* 12*c* unsaturation. After two years storage of the seeds the extracted oil contains about 6% of epoxy acids and a similar amount of hydroxy acids. Among these are oxygenated derivatives of the unusual triene acid including its 9,10-epoxide and 9- and 13-hydroxy derivatives[40] Freshly harvested soyabeans furnish an oil with about 0.3% of oxygenated acids. After only 1-2 months storage the value has risen to 1-2%[53]. About one half comprises 9,10-epoxy acids derived from oleic, linoleic, and linolenic acids and one third are hydroxy acids including mainly 9,12- and 13-hydroxy C_{18} diene acids with 9- and 12-hydroxy C_{18} monoene acids as minor components.

Hydroxy Fatty Acids

Natural long-chain hydroxy acids have been known to be seed oil constituents and these are categorised into three groups. One group possesses the hydroxyl function at

or near the carboxyl or methyl end of the chain, whilst those with mid-chain hydroxyl groups can be subdivided into acids with or without conjugated unsaturation. The end-chain hydroxyl acids are most likely to have this oxygenated function α or β with respect to the carboxyl group or ω_1 and ω_2 at the methyl end. Such acids are present in lipids derived from brain, wool, seeds, yeast, and cutins. Some α-hydroxyl acids occurring in seed oils[54-56] and α-hydroxy derivatives of acids such as oleic, linoleic, linolenic, or sterculic, with which they usually co-occur (e.g. α-hydroxy linolenic[54], D-2-hydroxysterculic acid[55]). It is of further interest that they may be also be accompanied by unsaturated acids with one less carbon atom. For example, *Salvia nilotica* (Labiatae) seed oil contains oleic, linoleic, and linolenic acids, their C_{17} analogues, and the α-hydroxy C_{18} acids[56]. It is reasonable to conjecture and there is some evidence for this that the α-hydroxy acids are intermediates in a chain-shortening process occurring by α-oxidation. The C_{17} acids have unsaturation in the position expected on this basis: the triene acids, for example, is $\Delta^{32,35,39}$.

C_{18} mid-chain hydroxy acids without conjugated unsaturation appear to be hydration products of oleic, linoleic, or linolenic acid. Hydration of an unsaturated alkene can, of course, yield two hydroxy compounds but the natural process under enzymic control could well be

regiospecific. Prominent among these acids are recinoleic [D-(+)-12-hydroxyoctadec-*cis*-9-enoic] acid in castor oil as a major component, and strophanthus (or *iso*-ricinoleic) an isomer of recinoleic acid (9-hydroxyoctadec-*cis*-12-enoic) which was first reported by Gunstone in several strophanthus oils[57].

The occurrence of ricinoleic acid has been reported as a major component in the seed oil of *Hiptage benghalensis*[58] while isoricinoleic acid as a major component in the seed oils of *Wrightia tinctoria*[59], *W. tomentosa*[59] and *W. coccinea*[60]. The higher homologues of ricinoleic and densipolic acids, lesquerolic [(+)-14-hydroxyeicos-*cis*-11-enoic] and auricolic (14-hydroxyeicosa-*cis*-11-*cis*-17-dienoic) acids have been discovered. Apart from the structural resemblance between these C_{18}/C_{20} pairs, the compounds usually co-exist suggesting that they are biosynthetically related. Further examples of chain extension are to be found among the epoxy and furanoid acids and other cases probably await discovery.

Mid-chain hydroxy acids with conjugated unsaturation can be further categorised into those which contain and those which do not contain acetylenic unsaturation. Smith *et al.*[54] found and characterized 9-hydroxy octadeca-*trans*-10, *trans*-12-dienoic acid, which they named dimorphecolic acid. Morris *et al.*[61] Chisholm and

Hopkins[62] found independently the mixture of 9-hydroxyocta deca-10,12-dienoic and 13-hydroxyoctadeca, 9-11-dienoic acids in seed oils. These acids were known to be either *cis*-, *trans* or *trans*, *cis* in configuration. Those acids with unsaturation which is entirely olefinic resemble the products of oxidation of polyene acids such as linoleic acid. By chemical or enzymic reaction, linoleic acid furnishes hydroperoxides at C9 or C13 with a double bond shifting into conjugation i.e. 9-hydroperoxy 10*t* 12*c* diene and the 13-hydroxperoxy 9*c* 11*t* diene. The initially formed *cis-trans* dienes pass easily to the *trans-trans* isomers.

Hydroxy acetylene acids tend to occur in fairly large proportions in some of the species that have acetylenic acids. Ligthelm[63] isolated 8-hydroxyximenynic acid from *Ximenia* oil. Miller *et al.*[64] in 1977 isolated two new hydroxy acetylenic acids 8-hydroxyoctadeca-10,12-diynoic and 8-hydroxyoctadec-17-en-10,12-dienoic acids from the same seed oil. Powell et.al.[65] characterized helenynolic acid, i.e. 9-hydroxyoctadec-*trans*-10-en-12-ynoic acid, an acetylenic analogue of dimorphecolic acid from *Helichrysum* seed oil. Powell *et al.*[66,67] detected and identified several new hydroxy acetylenic acids in *Acanthosyris* oil, of santalaceae family. One of these is the first C_{17} fatty acid (7-hydroxyhepta deca-*trans*-10, *trans*-16-dien-8-ynoic) to be found in quantity in a seed oil[68]. The C_{18} and C_{17} homologues may be related through α-oxidation though the

α-hydroxy intermediates have not yet been discovered. It appears that hydroxyacetylenic acids are nearly as numerous asd hydroxyolefinic acids in seed oils but may not occur in as many plant families.

There are three modes of occurrence of the long-chain hydroxy acids. Some, like ricinoleic acid in castor oil, from triacylglycerols in the conventional manner so that each glyceride molecule contains only three ester linkages. Other such as *Kamala* oil[69] and *Lesquerella auriculata*[70] seed oil generate glycerol esters with four ester linkages. Additional bonding results from acylation of the fatty acid hydroxyl group with a further long-chain molecule. The third category contains some of their hydroxy acids in lactone form viz. (s)-13-hydroxy octa deca-*cis*-9, *trans*-11-dienoic acid lactone (coriolide) from *Monnina emarginata* seed oil[71]. The lactone rings are of moderate size[71].

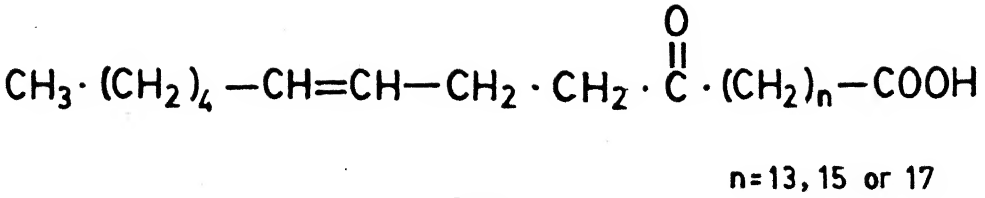
Koegh and Zurita[72] isolated a very unusual fatty acid and characterized it as α-(15-hydroxyhexadecyl) itaconic acid from a lichen *Ushnea aliphatica*. Later Keogh and Duran[73] isolated another new acid from *Ushnea meridensis* and characterized it as methyl 3,4-dicarboxy-3-hydroxy-19-oxoeicosanoate. Rukmini[74] isolated a solid acid from *Argemone maxicana* seed oil and structure of the acid was characterized as (+)-6-hydroxy-6-methyl-9-oxooctacosanoic

acid. However, Gunstone *et al.*[75] showed it to be a mixture of three oxo acids: 9- and 11-oxooctadosanoic and the oxotriacontanoic in an approximate ratio of 1:2:1. Two more new dihydroxy fatty acids (nonvicinal) were reported by Osman *et al.*[76] from seed oil of *Peganum harmala* and *Baliospermum axillare* and characterized as 9,14-dihydroxyoctadecanoic acid and 11,13-dihydroxytetracos-*trans*-9-enoic acid respectively.

Oxo Fatty Acids

Natural oxo or keto fatty acids are much less common. The oxo acids of plant origin are a rather heterogeneous group with no unifying features other than possession of one carbonyl group. Thus far, no fatty acids with more than one ketone function have been discovered. In *Oiticica oil* the conjugated triene acid, eleostearic (18:3, 9*c*, 11*t*. 13*t*), is accompanied by its 4-oxo derivative whilst *Chrysobalanus icaco* seed oil also contains the conjugated tetraene acid-parinaric (18:4, 9*c*, 11*t*, 13*t*,15*c*)- and its 4-oxo derivative[39].

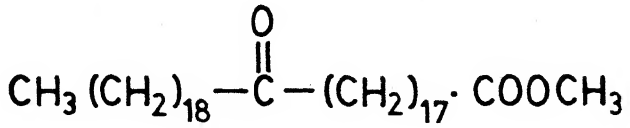
Two oil seeds contain mid-chain oxo acids of longer than usual chain length. *Cuspidara pterocarpa* seed oil contains C_{24}, C_{26} and C_{28} acids[77] all of which have the common C_{10} end-group (X). They could therefore arise from linoleic acid by a selective oxidation of C-9 followed by chain extension.



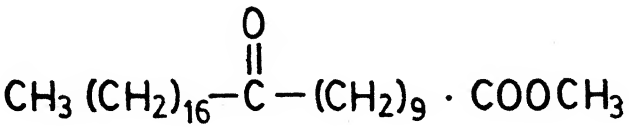
n=13, 15 or 17

[X]

Argcmone maxicana seed oil has been shown by Gunstone *et al.*[75] to contain three oxo acids with 28 and 30 carbon-atoms (XI, XII and XIII).



[XI]



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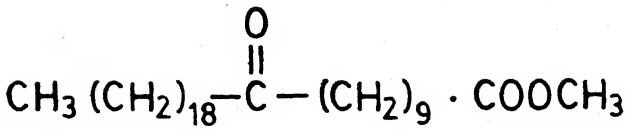


[XIII]

It is conceivable that these arise biologically from stearic and arachidic acids by a chain-extension process in which the original carbon remains in the oxidized form.

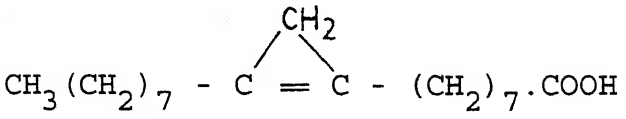
Alicyclic-substituted Fatty Acids

Hitherto three types of alicyclic-substituted acids have been encountered in natural fats, which are (i) Cyclopropane (ii) Cyclopropene and (iii) Cyclopentenyl (or Cyclopent-2-ene) acids.

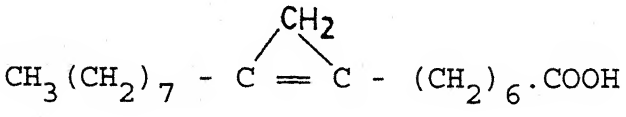
Cyclopropane and cyclopropene acids are characterized by the presence of three-membered saturated and unsaturated rings respectively at or near the centre of the hydrocarbon chain. Lactobcillic acid was the first cyclopropane fatty acid to be found in nature, other have now been discovered. The cyclopropane fatty acids are commonly produced by the microorganism but they are also found generally in small amounts in certain seed oils where they may be biosynthetic precursors of cyclopropene fatty acids. Dihydrosterculic acid is a major constituent (17.4%) of the seed oil of *Dimocarpus longans* (*Euphoria longana*)[78] of sapindaceae family and it is also accompanies the cyclopropene fatty acid, sterculic acid, in many species of the order Malvales.

Cyclopropene fatty acids have been found mainly in seed lipids, though they also exist in other tissues of four plant families of the order Malvales (Sterculaceae, Malvaceae, Bombaceae and Tiliaceae) and may be accompanied by small amounts of the saturated analogue of sterculic acid, dihydrosterculic acid. The sterculic acid was first isolated by Nunn in 1952[79] from *Sterculia foetida* seed oil

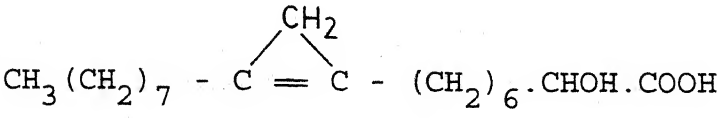
and showed it to be 9,10-methyleneoctadec-9-enoic acid (XIV). Shortly afterward malvalic acid (8,9-methylenehepta-dec-8-enoic acid (XV) was isolated and characterized[80-81] and two more cyclopropene fatty acids have been discovered. D-2-hydroxysterculic acid (XVI)[55] and sterculynic acid (8,9-methyleneoctadec-8-ene-17-ynoic acid (XVII)[82]. Osman *et al.* in series of publications[83-84] showed that seed oils of *sida acuta, S. rhombifolia, S. grewioides, Hibiscus sabdariffa, Abutilon indicum, Urena, lobata,* and *Eriolaena hookeriana*, were found to contain sterculic and malvalic acids as unusual constituents of triglycerides in addition to conventional fatty acids.



[XIV]



[XV]



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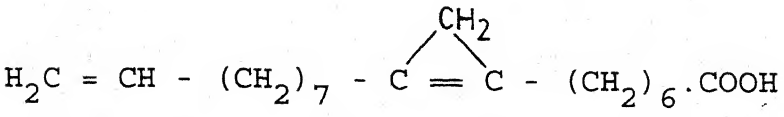
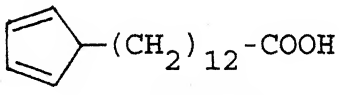
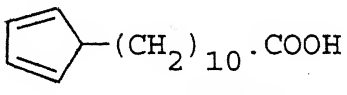


[XVII]

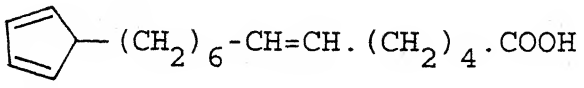
The fixed oils expressed from the seeds of most of the members of *Hydnocarpus* genus of the Flacourtiaceae family are commonly known as chaulmoogra oil and are used extensively in the treatment of leprosy and other cutaneous diseases. These oils are characterized by the presence, in predominating amount of unsaturated cyclic fatty acids mainly hydnocarpic, chaulmoogric and garlic acids, which do not seem to occur in any other seed oils than those of Flacourtiaceae family. All of these oils contain one or more fatty acids having a terminal cyclopentene ring, especially chaulmoogric (XVIII), hydnocarpic (XIX), and garlic (XX) acids, which account for 80-90% or more of the total fatty acids of these oils.



[XVIII)



[XIX)



[XX]

Other homologues namely, alepric, aleprylic, alepristic, and aleprolic acids were found to be present in small proportions in some of these oils.

All the evidences of the past years suggest that nature still has some surprise for us in terms of fatty acid structure.

Isolation and Characterization of Fatty Acids

A survey of the literature indicated that most of the analysis of vegetable seed oils from Indian species were based on the classical methods of oil analysis. Screening analysis of seed oils containing unusual functional groups often gave unexpected responses to analytical procedures in frequent use. Chromatographic and spectroscopic instrumentation have greatly shortened the time needed for organic chemical studies and have increased accuracy in judging homogeneity and purity of materials manifold. The innovations of chromatographic techniques, particularly thin-layer and gas-liquid chromatography, together with advances in spectroscopy, have led to elucidation of many new fatty acid structures and revision of certain others reported earlier. The main corrections of old mistakes continue to occur in stereochemistry since organic chemists have learnt to determine absolute configurations and preferred conformations by roentgenographic analysis, by NMR spectroscopy and by measurements of circular dichroism.

In seventies, a few diagnostic spot tests have been developed for the detection of unusual fatty acids. Some spot reagents include, picric acid for epoxy acids[85]; 4-(p-nitrobenzyl) pyridine for hydroxyl[86] and acetylenic functions[87]; 4-amino-5-hydrazino-1,2,4-triazole-3-thiol for aldehyde group[88]; 2,4-dinitrophenylhydrazine for keto[89]; ferrous thiocyanate for hydroperoxide[90] and Halphen's

spraying reagent for cyclopropanoid acids[54].

The assaying of fatty acids has experienced a total revolution with the development of chromatographic techniques. First developed by the use of adsorption column chromatography[91], argentation[92-95] and gas-liquid chromatographic techniques were then rapidly adopted for the analysis of fatty acids. The chemical identification of the fatty acids by chromatography is based exclusively on the similarity of R_f values or retention time with those of reference compounds of known structures. Unfortunately, because of the lack of specificity of these techniques, such criteria are insufficient proof of the chemical identity of a plant product and more specific information such as IR, NMR and MS data are essential requirements for unequivocal establishment of chemical structure.

Modern separation and purification techniques, such as chromatography and electrophoresis have tremendously facilitated isolation. A phenomenal separation method recently adopted in lipid chemistry is thin-layer-chromatography (TLC).

Some interesting new approaches involve use of complexing agent during TLC of fatty acids. Impregnation of TLC adsorbents with silver nitrate to aid resolution of *cis* and *trans* isomers and higher unsaturates has been suggested by various workers[94-97]. Morris[92,96] also proposed the

separation of mono, di and poly-hydroxy acid esters. *Threo* and *erythro* forms of various vicinal diols may be separated by the use of boric acid-TLC[96].

In the late fifties, the introduction of gas-liquid-chromatography (GLC) along with TLC revolutionized lipid chemistry. Indeed, it is difficult to imagine lipid research without these now indispensable techniques. GLC now probably most widely applied method for structural analysis of fatty acids, including polyunsaturated fatty acids (PUFA)[97-99], and is often applied in conjunction with mass spectrometry. Though GLC has brought to the oil chemists a more useful and versatile analytical tool than any which proceeded it, several examples will suffice to show some limitations of this technique in application to many types of samples.

The use of preparative GLC has made it possible the isolation of pure fractions from a complex mixture. The collected fractions can be chemically modified and then re-examined by chromatographic techniques. Keto compounds can be converted to N,N-dimethyl hydrazides, or reduced to hydroxy compounds. Hydroxy esters can be oxidized to keto esters, acetylated or converted to their trimethylsilyl ethers, trifluoroacetyl, or isopropylidine derivatives.

Spectroscopy aids to the recognition and location of functional groups in fatty acid chains. Sometime, the

structure can be completely recognized by the use of appropriate spectroscopic techniques with a choice between ultraviolet, infrared, nuclear magnetic resonance (^{1}H NMR with or without chemical shift reagent, and ^{13}C NMR) and mass spectroscopy. In some cases it is desirable to hydrogenate the acid first, to identify the perhydro derivative and then to tackle the problem related to unsaturation - its nature, configuration, and position.

UV spectroscopy is useful primarily as a means of detecting conjugation in polyunsaturated fatty acids. A direct UV spectrum of an oil showing maxima in the region 200-400 nm gives positive indication for the presence of conjugation. UV determines the number, and to some extent, the kind of multiple bonds in conjugation. As the number of multiple bonds in conjugation increases, UV absorption maxima occur at progressively longer wavelengths[100-102]. For example, dimorphecolic (9-hydroxyoctadeca-*trans*-10, *trans*-12-dienoic) shows absorption maxima in methanol at 231 nm[100], β-eleostearic (octadeca-*trans*-9, *trans*-11, *trans*-13-trienoic) gives λ_{max} ethanol = 259, 268 and 279 nm[100].

In the past, UV has been applied to the structural analysis of PUFA mainly conjunction with isomerization procedures. PUFA were analyzed by UV after treatment under rigorous alkaline conditions ("alkali isomerization"). The

number of double bonds in the original acid could be deduced by this procedure provided there was no deviation from methylene-interrupted spacing of double bonds in the starting material. This method is now less frequently used since the advent of GLC.

IR spectroscopy is a considerably more versatile tool for structural analysis. IR spectroscopy is of particular value in the recognition of unusual functional groups and in detecting and measuring *trans* unsaturation in fatty acids. One *trans* bond produces characteristic absorption at 970 cm^{-1}. For non-conjugated polyenoic acids, the effect is roughly additive so that linelaidic acid will have an absorption band at the same position as elaidic acid with increased intensity. Conjugated polyene systems with one more *trans* bond show a shift in the position of adsorption. Other particular values in the detection of unusual functional groups are 1724 (carbonyl), 3448 (hydroxyl), 1852 and 1010 (cyclopropenoid), 826 and 848 (epoxide), 2222 and 1061 (allene), 952 (conjugated enyne system) and 2240 (nitrile group) cm^{-1}. The use of NMR and mass spectroscopies in the structural determination of fatty acids is described in Part-II of the thesis.

Ideally, individual pure fatty acids (usually in the form of the methyl ester derivatives) should be isolated by chromatographic procedures and examined first by

non-destructive spectroscopic tecniques before chemical degradative procedures are applied. For example, adsorption chromatography will separate normal fatty acids from those containing polar functional groups. Silver nitrate chromatography can be used to seggregate fatty acids according to the number and geometrical configurations of their double bonds; a portion of each fraction should be hydrogenated so that the lengths of the carbon chain of the components can be confirmed. The position and configuration of double bond(s) may be determined by spectroscopic (principally IR and NMR spectroscopy) and oxidative degradation procedures. Appropriate spectroscopic and chemical techniques must also be used to detect and locate any other functional groups.

Epoxides are indicated by their TLC and GLC behaviour and confirmed by IR and NMR spectroscopic studies. The position of an epoxide group can also be determined by mass spectroscopy of the epoxy ester or after converting them into a number of derivatives including the o-methyl, o-trimethylsilyl ether. The readiness with which epoxides undergo cleavage directly with periodic acid or after ring opening to the diol, is the basis of a simple degradation procedure.

The chemical methods are to be employed in almost all the cases for an unambiguous characterization of the fatty acids, inspite of the development of chromatographic

and spectroscopic techniques. The chemical methods generally used are hydrogenation, hydroxylation, partial hydrogenation, oxidation and partial oxidation, hydrogen bromide reaction, addition of dienophiles like maleic anhydride (Diels-Alder reaction). Besides these reactions some specific procedures have been found to be more useful for solving special types of structural problems. These include hydrogen bromide titration of oil before and after reduction by $LiAlH_4$, cleavage of saturated hydroxy acid by solid potassium permagnate, dehydration of a dienol to all *trans* triene acids by treatment with glacial acetic acid, reduction of secondary alcoholic groups -CHOH to $-CH_2-$ by HI/P, reductive removal of hydroxyl group by the reduction of the tosylate with $LiAlH_4$ followed by oxidative degradation of unsaturated acid by permagnate periodate and lipoxidase catalyzed isomerization to conjugated fatty acids.

B♦ PRESENT WORK

1. Cyanolipids of Sapindaceous and Borage Seed Oils

Three sapindaceous seed oils, viz. *sapindus saponaria, S. trifoliatus, Lepisanthes tetraphylla* along with four boraginaceae seed oils namely *Cordia obliqua (C. wallichii), C. dichotoma, Heliotropium indicum* and *H. eichwaldi* were investigated for their nitrogen-containing-lipid fraction (NCLF).

A correction work of David S. Seigler[103] has reported that the seeds of the material previously identified as *Cordia verbinacea*, were from a sapindaceous plant probably of the genus *Allophyllus*. The anomalous occurrence of cyanolipid in this member of boraginaceae is thus resolved. The *Heliotropium* species reported by I. Ahmad[104] *et al.* to contain cyanolipid were reinvestigated by us.

The present work on *Heliotropium indicum, H. eichwaldi, cordia obliqua (C. wallichii), C. dichotoma*, all of boraginaceae family revealed the absence of cyanolipid in all these four species. Thus the distribution of cyanolipid seems to be limited to member of sapindaceae family only.

In present investigation we have isolated and characterized this new class of lipid in *sapindus saponaria, S. trifoliatus and Lepisanthes tetraphylla* seed oils. *L.*

tetraphylla seed oil was found to contain a fatty acid diester of 1-cyano-2-hydroxymethylprop-2-ene-1-ol (**V**, 22%). *S. saponaria* and *S. trifoliatus* seed oils were however found to contain a fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol in varying proportions (**VI**, 15, 20%).

On silica gel TLC the seed oil of *L. tetraphylla* gave spot of cyanolipid slightly ahead of triglyceride with ether-hexane (1:3) and two spots in benzene (triglyceride, R_f 0.53 and cyanolipid R_f 0.72). The oils from *S. saponaria* and *S. trifoliatus* gave two spots (triglyceride, R_f 0.86 and cyanolipid R_f 0.60) with ether-hexane (1:3) and only a single spot with benzene. The Nitrogen-Containing-Lipid-Fractions (NCLF) were separated from triglyceride fractions in a pure state by preparative TLC. For NCLF (I) separation with benzene was done and for NCLF (II) the plate was developed with ether-hexane (1:2).

Analysis of Nitrogen-Containing-Lipid-Fraction (NCLF-I)

Bands observed at 938 and 1015 cm^{-1} with IR spectrum of the oil were intensified in the spectrum of NCLF. The absence of nitrile absorption in the IR spectrum of a cyanolipid ester such as (I) is consistent with previous observations. The characteristic - C≡N absorption near 2250 cm^{-1} is likely to be quenched if an oxygen is

joined to the carbon bearing the nitrile group[105] and the IR spectrum was superimposable on spectrum of corresponding cyanolipid isolated from *Cardiospermum halicacabum* seed oil.

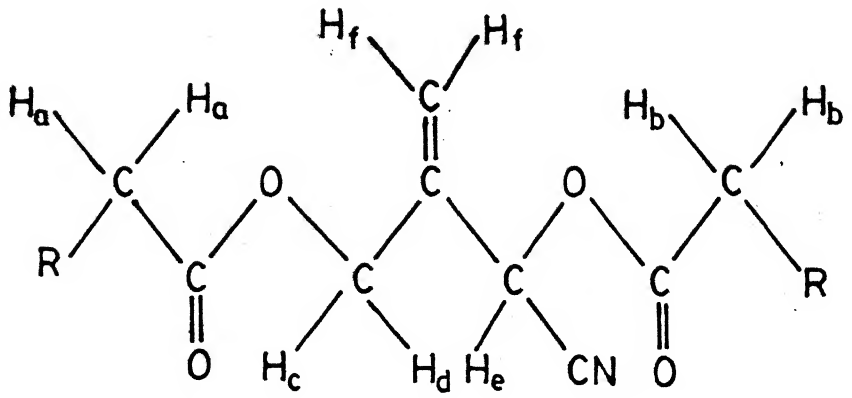
Elemental analysis of NCLF indicated that it contained 2.1% of nitrogen by Dumas method.

Treatment of NCLF with dilute base, generated HCN as shown by positive picrate and prussian blue tests.

Methyl esters from NCLF I had the composition as shown in Table II.

The NMR spectrum of the NCLF (I) in $CDCl_3$ showed signals of τ 9.12 (terminal methyl protons, 6H), τ 8.75 (shielded methylene protons), τ 8.05 (allylic protons), τ 4.7 (olifinic protons). The signal centered at τ 7.66 (4H) in the $CDCl_3$ spectrum appears in benzene-d_6 as a pair of overlapping triplet of τ 7.64 and 7.68 and is due to non-equivalent methylene protons to fatty acid carboxylic groups. Besides these signals, addition peaks appeared at τ 5.35 (2H) which is resolved in benzene-d_6 into overlapping doublets centered at τ 5.44 and 5.60; this observation demonstrate that the geminal methylene protons H_c and H_d of the dihydroxy nitrile moiety are also non-equivalent. The farthest down field signal at τ 4.06 is assigned to proton H_e attached to the cyanohydrin carbon. The remaining

signals at τ 4.34 and τ 4.50 arise from protons H_f and H_g in structure **Va**.



(Va)

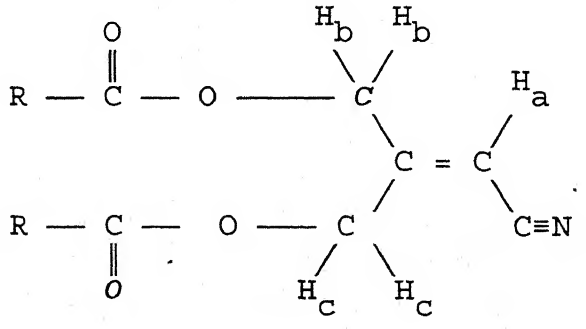
(Cyanolipid (NCLF I) separated from *L. tetraphylla* seed oil)

Analysis of Nitrogen-Containing-Lipid-Fraction NCLF-II

The IR (1% solution in (CS_2) analysis of NCLF (II), **(St. VIa)** revealed a band of medium intensity of 2200 cm^{-1} (C≡N) group and the IR spectra were superimposable on the spectrum of corresponding cyanolipid isolated from *S. emarginatus* seed oil.

The NMR spectra of the cyanolipid revealed proton counts, chemical shifts and multiplicities identical with those displayed by the reference sample. The NMR exhibited signals characteristic for long chain lipid group τ 9.12 (rough t, 6H, terminal methyl), τ 8.75 (br S, shielded

methylene), τ 7.97 - 8.05 (m, protons α to double bond), τ 7.67 (t, protons α to the carbonyl function), and at τ 4.7 (rough t, olefinic protons). The two sets of methylene protons (Hb) and (Hc) (**VIa**) which are adjacent to the oxygen atoms of the dihydroxynitrile moiety gave the signals at 5.83 (singlet) and 5.93 (doublet). This difference in shielding is caused by the stereochemistry of the methylene groups; one of them is *cis* to the nitrile grouping and the other is *trans*. As a result of the stereochemical difference between the two methylene groups, the protons of one group couple more strongly with the vinyl proton (τ 4.45) than to protons of the other methylene group. The cyanohydrin proton (H_a) appeared as a slightly broadened singlet at τ 4.45. The comparative TLC and IR characteristics coupled with NMR data established that the cyanolipid present in the oil is a fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol identical to the NCLF of S. *emarginatus*.



(VIa)

(Cyanolipid Separated from *Sapindus saponario* and *S. trifoliatus*)

The lipid groups of the triglycerides as well as cyanolipid constituent of the oil (Table II) were identified by converting them to their methyl esters by transesterification or acid-catalyzed methylation and comparing the methyl esters by GLC with authentic standards. On comparing it was found that a higher proportion of C_{20} acids occur in the cyanolipids than in the triglycerides.

Because of its basically isoprenoid structure, the dihydroxynitrile moiety of NCLF has many biogenetic possibilities. It may be related, perhaps somewhat remotely to biological compounds such as cordy cepose[106] or mevaldic acid[107]. However, rather extensive studies made on biosynthesis of other cyanogenetic materials[108-110] indicate that most of the them are derived from amino acids or their precursors.

Experimental Procedures

(i) Source of oil seeds: The seeds used as source of oils, for the present study, were procured from various sources, including National Botanical Research Institute, Lucknow, Oil and Fats Department, AMU, Aligarh, established commercial suppliers viz. Partap Nursery and Seed Stores, Dehradun and United Chemicals and Allied Products, Clive Row, Calcutta.

(ii) Oil extraction: Cleaned, dry samples of seeds were usually ground in a disintegrator. The powdered seeds were extracted exhaustively with petroleum ether (bp 40-60^{o}) in a soxhlet apparatus until no more oil was available. The solvent was removed at reduced pressure under nitrogen to find out the oil content of the seeds. The crude oil was neutralized by passing it (~ 1 g) in chloroform solution, through a short column of alumina (~ 10 g). Seed oil properties viz. moisture content, iodine value, saponification value, refractive index determined by AOCS methods[113].

(iii) Preparation of mixed fatty acids: Seed oil was refluxed with ethanolic potassium hydroxide. The unsaponifiable matter was removed and free fatty acids were obtained in the usual manner. Wherever necessary, saponification was carried out under nitrogen and samples

were stored at low temperature in a nitrogen atmosphere.

(iv) Preparation of methyl esters: Esterifications were carried out as follows, except where specified. Fatty acid samples were refluxed for 1 hr in a large excess of absolute methanol containing 1% conc. sulphuric acid (V/V). In each case, resulting mixture diluted to the cloud point with water, chilled in ice-bath, and then extracted repeatedly with ether. Combined extracts were dried over sodium sulphate, and filtered and the solvent was removed under reduced pressure.

(v) Thin-layer-chromatography 'TLC': Thin-layer-chromatography was done on 0.25 mm layers of silica Gel G developed with solvent system of benzene or hexene-ether-acetic acid (85 : 15 : 1, V/V/V). Spots were visualized by keeping TLC plates in iodine-chamber for sometimes 15-30 minutes. The oil was resolved into a triglyceride fraction and a cyanolipid fraction by preparative TLC. For preparative TLC separation plates 29x49 cm with silica Gel G layers, 1 mm thick were used. The solvent was ether-hexane (1:2, V/V). The spots were detected by spraying with an alcoholic solution of 2,7-dichlorofluorescein and viewing them under ultraviolet light. Desired constituents were recovered from

the silica by standard procedures and the purity of the fraction was checked by analytical TLC.

(vi) Infra red: IR spectra were determined with Perkin-Elmer Model 1320 spectrophotometer as liquid film or as 1% solution in carbon disulphide or carbon tetrachloride.

(vii) Ultraviolet: A Beckman DK-2A instrument was used to determine UV spectra in methanol.

(viii) Gas-liquid chromatography: GLC analyses of methyl esters were performed essentially as described by Miwa and coworker[114] by using stainless steel packed column (2x3 mm) coated with diethylene glycol succinate (DEGS), 15% on chromosorb, W, 45-60 mesh). A Perkin-Elmer Model 154 vapour Fractometer was employed in these analyses and the separations were carried out isothermally at 200°C, with a hydrogen flow rate of 70 ml/min. The methyl ester of linseed oil was used as an standard for internal standardization.

(iv) Formation and detection of HCN: In the present investigation two tests were used to detect HCN derived from seed oil and nitrogen-containing-lipid fraction (NCLF). One of these, the picrate test depends on the reaction of HCN

with alkaline picrate solution to produce isopurpuric acid[115]. About 100 mg of lipid material was placed in a test tube with 1 ml of dilute NaOH or H_2SO_4. A filter paper strip dipped in an alkaline solution of sodium picrate (0.5%) was partially dried and was then suspended over the mixture in the stoppered test tube and contents were warmed at 35-50^{o} for 30-60 minutes. A positive test involves a colour change of the filter paper strip from yellow to brick red[116].

The second test involved formation of prussian blue[117]. Material to be tested was placed in 50 ml Erlenmeyer flask with 2 ml of methanol and either 1 ml of 10% of NaOH or 1 ml of 6N H_2SO_4. If NaOH was used, the mixture was heated a few minutes in a hot water bath and acidified with H_2SO_4. A strip of filter paper moistened with NaOH solution was placed over the mouth of the flask and the flask was warmed for 5-10 min. After the filter paper was removed, it was treated with three drops of 5% ferrous sulphate solution and, when nearly dry, with 10%, HCL. An intense prussian blue colour indicated a positive test for cyanide.

2. Fatty Acid Analysis of Indigenous Seed Oils

Upto sixties our knowledge of composition, structure and location of lipids were meagre indeed. The recent major developments have been due mainly to innovation of new analytical and chromatographic techniques. The older methods of studying fatty acid composition of oils were inadequate to detect very minor components of fatty acids. A survey of the literature on seed oil composition indicates that most of the oil analyzed earlier by classical methods are now found to contain less familiar acids possessing a variety of functional group. The recent developments have been due mainly to the availability of new analytical and chromatographic techniques.

Vegetable oils are the second most important group of cereals amongst the agricultural commodities and they constitute an important part of human diet besides their various industrial applications. As a result the demands for vegetable oils has been rapidly increasing with improvement in general standard of living, increase in population and technological advances, but the supply has not increased in the same proportion thus creating increasing gap. It is well known that we are an edible oil deficient country. The par-capita availability of oils and fats is only 5 kg compared to atleast 22 kg recommended by nutritionists. From being an oil exporting country in 1960

India today has become seriously dependent on edible oil imports. The acute scarcity and rising prices of vegetable oils for edible purposes and industrial use had stimulated research in the screening of oil-bearing seeds from wild plants for finding non-traditional sources of vegetable oils. It now realized that systematic screening of indigenous seed oils may discover oils containing either a high concentration of one of the common natural fatty acids or less common or unknown acids having a structure of scientific interest.

In the present work the preliminary studies on oil analysis revealed that no significant amount of unusual fatty acids were present. GLC studies have shown that these oils are composed of common fatty acids but in varying proportions. The fatty acid composition of triglycerides and NCLF is reported in Table-II. The oil content of the sapindaceous seeds was quite variable and ranged from 18.5% in *Lepisanthes tetraphylla* to 32% in *sapindus saponaria* whereas borage seeds ranged from 6.8-25.5%. The iodine values of oils ranged from 125.5 - 130 (Table-I). In the present investigation three oil seeds belonging to sapindaceae and four oil seeds of boraginaceae family have been analyzed for their fatty acid composition of triglyceride and NCLF by using chromatographic and

spectroscopic techniques, while the possibility for the presence of unusual character like conjugated polyunsaturation and *trans*-unsaturation was ruled out with the help of UV and IR studies. Various TLC techniques showed the absence of oxygenated or unusual functional group. Esters gave clear spots on argentation TLC[111] corresponding to the saturated monoene, diene and triene (Table-II) parallel to those from authentic linseed ester resolved along side.

The presence of C_{20} acids (saturated and monoene) was detected in all the three seeds of sapindaceae found to contain cyanolipid fraction (Item 1-3, Table-II), whereas the presence of C_{16} and C_{18} saturated acids were detected in all the species including the cyanolipid fractions in *S. saponaria, S. trifoliatus* and *L. tetraphylla*. The presence of C_{22} saturated acids in some species in small amount was established with the help of reversed phase TLC[112]. The quantitation of fatty acids as their methyl ester was carried out by GLC analysis by using both polar and nonpolar columns (DEGS, 15% and SE, 2%) and by measuring the peak area by integrated method. The identification of each acid was made by comparing its retention time with that of standard sample run under the same condition. The result of quantitation direct, reversed phase and argentation TLC

supported by the findings of GLC analyses.

The total content of saturated acids ranged from 8.3-38.2%. All the seven seed oils contained palmitic and stearic acids, combined content ranging 5.1 - 22.7% . Seed oil of *Heliotropium eichwaldiv* (Item-5, Table-II) and *H. indicum* (Item-4, Table-II) were found to contain palmitic acid as high as 18.3 and 18.5 respectively. The stearic acid was present to the maximum of 7.1% in *H. eichwaldi* (Item-5, Table-II). In the combined content of palmitic acid and stearic acid, palmitic was found to be present as a major component in all samples, which is the unusual pattern of distribution of palmitic and stearic acid. Other than C_{16} and C_{18} saturated acids C_{20} and C_{22} saturated acids were also found to be present in various species in minor amounts. C_{22} monoene acids (Behenic) was found to be occurring in three species only (Item 1,3 and 7, Table-II) to the extent of 12-3.5%. The oil of *H. eichwaldi* contained the maximum amount of saturated acids (25.4%). The total of unsaturated acids in the species examined varied from 66.8 - 91.7 in the triglyceride fractions. C_{18}-unsaturated acids ranged from 50.4 - 89.9%. Among C_{18}-unsaturated acids oleic and linoleic acids were found to be the most frequently occurring acids rather than linolenic acid which was present as minor constituent ranging from trace amount to 06.1%.

The combined content of oleic and linoleic acid was found to vary in the region 50.4 - 83.8%. *Cordia dichotoma* and *C. obliqua* (*C. walichii*) contain 76.9% and 83.8% of oleic and linoleic acid respectively. A moderately high percentage 21.4% of C_{20} monoenoic acid (eicos-11-enoic) was found to be occurring in *L. tetraphylla* (Item-3, Table-II). Two species namely *H.* indicum and *H. eichwaldi* yielded moderately linoleic-rich seed oils containing 34.1 - 44.1% of linoleic acid. *C. obliqua* and *C. dichotoma* also contained a good amount of linoleic acid 21.6% and 23.2% respectively.

In cyanolipid fraction of *L. tetraphylla* linoleic acid falls to 1.8% of the total faty acids but monoenoic acid (oleic and eicos-*cis*-11-enoic) takes its place as the predominating components (48.6% oleic and eicos-*cis*-11-enoic, 21.4%). The eicos-*cis*-11-enoic acid content in nitrogen-containing-lipid-fraction of *L. tetraphylla* was found to be maximum among all three sapindaceous species reported in our present investigation. The characteristics and the fatty acid composition reported by previous workers are similar to our results.

Most of the oils belonging to sapindaceae family are rich in oleic acid. The triglyceride fraction and NCLF of all the three sapindaceous oil was found to be rich in oleic acid ranging from 48.6 - 62.6%. The linolenic acid

content of all the three species reported in the present investigation ranged from trace to 6.1%. The eicosanoic acid was found to be present in substantial amount in both the fraction (TG and NCLF) of sapindaceous seed oils ranging from 12.3 - 30.8%. The present investigation also revealed the preferential incorporation of C_{20} monoene acids in NCLF. Among the boraginaceae seed oils it was found to be absent or present in very meagre amount. The eicos-*cis*-11-enoic acid was found to be present in a good amount only in nitrogen-containing-lipid fraction of *L. tetraphylla* (21.4%). The nitrogen-containing lipid-fraction of other two species viz. *S. trifoliatus* and *S. saponaria* contained only 12.1 and 13.2% eicos-*cis*-11-enoic acid respectively. The triglyce ride fractions (TG) of these sapindaceous species also contained eicos-*cis*-11-enoic acid but ranging only from 4.3-9.5% which also indicates the preferential incorporation of C_{20} monoene in NCLF. A meagre amount of C_{20} monoene was also found to be present in *Cordia obliqua and C. dichotoma* (1.8% and 3.7% respectively). The presence of behenic acid in all the seven species was found to be present in trace or meagre amount upto the tune of 3.5% only in the nitrogen-containing-lipid-fraction of *L. tetraphylla.*

Oil from *H. eichwaldi* contained 44.1% of linoleic acid with no linolenic acid may be grouped as semidrying

oil, whilest *S. saponaria*, *S. trifoliatus*, *L. tetraphylla*, *H. indicum*, *C. obliqua* (*C. wallichii*) and *C. dichotoma* which contained less of linoleic acid (below 40%) and no or very small amount of linolenic acid belong to the group of non-drying oils. A serious consideration can be given to species rich in oils as well as in specific acids if any can meet the agronomic standards of a field crop.

TABLE I

Sl.No.	Plant Identity	Seed Analysis		Oil Properties			
		Moisture %	Oil Content %	Iodine value	Saponification value	Ref. Index n_D^{20}	Cyanolipid (%)
1.	*Sapindus saponaria* (Sapindaceae)	3	32	125.3	190.4	1.4868	II(15%
2.	*Sapindus trifoliatu* (Sapindaceae)	1.6	23	125.8	191.0	1.4800	II(20%
3.	*Lepisanthes tetraphylla* (Sapindaceae)	5	20	126.3	192.2	1.4774	I(22%
4.	*Heliotropium indicum* (Boraginaceae)	5.8	13.2	127.1	200.5	1.4712	-
5.	*Helitropium eichwaldi* (Boraginaceae)	6.1	25.5	126.6	198.7	1.4740	-
6.	*Cordia Obliqua (C.wallichii)* (Boraginaceae)	8	7.8	130.2	195.3	1.4820	-
7.	*Cordia dichotoma* (Boraginaceae)	8.6	6.8	128.7	193.0	1.4850	-

TABLE II

Sl. No.	PLANT IDENTITY	TG/ NCLF	Conc. %	Methyl Ester Composition of Triglyceride and Cyanolipids, % by GLC						
				16:1	18:0	18:1	18:2	18:3	20:0	20:1
1.	*Sapindus saponaria*	TG	85	11.9	2.3	53.9	6.3	2.6	14.0	7.8
	(Sapindaceae)	NCLF	15	4.7	1.2	48.8	1.3	trace	30.8	13.2
2.	*Sapindus trifoliatus*	TG	80	10.3	2.6	51.8	7.4	2.2	16.2	9.5
	(Sapindaceae)	NCLF	20	5.4	1.7	49.6	1.6	1.2	28.4	12.1
3.	*Lepisanthes tetraphylla*	TG	78	9.4	3.2	62.6	6.7	1.5	12.3	4.3
	(Sapindaceae)	NCLF	22	3.7	1.4	48.6	1.8	trace	19.6	21.4
4.	*Heliotropium* indicum (HATHI SURA) (Boraginaceae)	TG	100	18.5	4.2	41.9	34.1	1.3	-	-
		NCLF	NIL							
5.	*Heliotropium eichwaldi* (NEEL KATTI) (Boraginaceae)	TG	100	18.3	7.1	30.5	44.1	-	-	-
		NCLF	NIL							
6.	*Cordia obliqua* (*C. wallichii*) (Boraginaceae)	TG	100	5.1	3.2	62.2	21.6	6.1	trace	1.8
		NCLF	NIL							
7.	*Cordia dichotoma*	TG	100	6.2	4.6	53.7	23.2	5.7	1.7	3.7
	(Boraginaceae)	NCLF	NIL							

Oil Recovery and Methyl Ester Formation: Oil was recovered from finely ground seed by a 16 hr extraction with petroleum ether (bp 40-60°) in a soxhlet apparatus. The methyl esters were prepared using 1% NaOMe in methanol or acid-catalyzed methylation.

Apparatus: Infrared (IR) spectra were determined with Perkin-Elmer Model 1320 spectrophotometer on 1% solution in CS_2, $CHCl_3$ or CCl_4. Nuclear magnetic resonance (NMR) spectra were obtained with a JEOL PMX 60 spectrometer; the solvent used was CCl_4. All reported chemical shifts are measured from internal tetra methylsilane (TMS) = τ 10.0. A Beckman DK-2A instrument was used to determine UV spectra. GLC analyses methyl esters were carried out as described by Miwa *et al.*[114]. A Perkin-Elmer Model 154, equipped with thermal conductivity detector, using stainless steel packed column (2m x3mm) coated with diethyleneglycol succinate (DEGS, 15% as chromosorb, W, 45-60 mesh) and a 60 cm x 4 mm column of silicone (SE - 30, 2%). Temperature at the injection port, detector block and column were 290°, 260° and 190° respectively. Attenuation 4, bridge current 150 m amp., chart speed 0.75 m/hr with a hydrogen flow of 70 ml/min. Linseed oil methyl ester was used as an standard, for internal standardization. Pure sample of lipid

standards were purchased from sigma chemical company, U.S.A.

Thin-layer Chromatography: TLC analyses of the oil as well as the methyl esters were done on plates coated with 0.25 mm or 1.0 mm thick layers of silica Gel G or 20% silver nitrate-impregnated silica Gel G with 20% or 30% ether in hexane as the developing solvent. For reversed-phase TLC, the dried, coated plates were uniformly impregnated with silicone oil (E. Merck). Solvent system acetonitrile-acetic acid-water (70 : 10 : 20; V/V/V) was used for development. Layers of silica Gel G 1 mm thick and hexane-ether (85:15, V/V) were used for preparative TLC of the esters. Bands were visualized by keeping in the iodine-chamber for 15-30 min., then the separated components were recovered by the usual procedure.

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PART -II

REACTION OF NITROSYL BROMIDE WITH LONG-CHAIN FATTY ACIDS AND THEIR DERIVATIVES

A - THEORETICAL

A wide variety of reactions on fatty acids, especially with unsaturation have been carried out and we are gradually increasing our understanding and awareness in the mechanism of these reactions. At a simple level one might expect that any reaction observed on a short-chain acid or alkene should be applicable to long-chain substrates.

Reactions of fatty acids in general are associated with (i) the carboxyl group and (ii) the hydrocarbon chain. During the first quarter of present century very little was known about the mechanism and stereochemistry of reactions of double bond. With the growing understanding of the mechanism of organic reactions, the controversial problem of organic chemistry were gradually solved.

Among the reactions involving the hydrocarbon chain of fatty acids those of oxidation, hydrogenation and halogenation are of fundamental importance in lipid chemistry. The large variety of products resulting from these reactions of an unusual fatty acid has been the main drawback in its systematic study. The opportunities which exist require that extreme care should be taken in their preparation, isolation and in selecting the criteria of purity.

A survey of the literature reveals that the results obtained by different groups of workers at different times have led to the interpretations which are conflicting, as far as the mechanism and stereochemistry are concerned.

It is now realized that organic chemistry is, to a large extent, the study of reactions of functional groups with important contribution of polar, stearic, conformational and neighbouring groups effects. Only from seventies onwards, new and interesting reactions of fatty acids have been described that provide new route to the synthesis of a variety of fatty acid derivatives. The growing demand of fatty chemicals as intermediate raw materials has diverted the attention of lipid chemists from the analytical aspect of fats to the chemistry of unusual fatty acids.

Recent developments in the lipid chemistry begin from 1960 to date. This period is characterised by a series of investigations on the non-classical reactions of fatty acids. These non-classical reactions include oxymercuration, demercuration, rearrangement of 1,2-epoxide, cyclodehydration (1,4-epoxide) of hydroxy olefinic acids, allylic halogenation and oxidation of olefinic acids, cyclopropanation and reactions leading to the synthesis of nitrogen and sulphur analogues of the oxygenated acids. The growth of organic nitrogen chemistry has been rapid, and it

not only shows no sign of abatement, but the literature has been proliferating at an increasing rate. Acids from the intellectual challenge involved in preparing novel organic nitrogen compounds of the most amazing complexity and structure ramification, organic chemists should be deeply concerned with nitrogen compounds because of their widespread use and intrinsic importance.

Nitrosyl halide (NOCl and NOBr) addition represents one of the simplest way of elaborating a carbon-nitrogen bond directly from unsaturated compounds. The reaction of nitrosyl chloride and nitrosyl bromide with olefins has been known for almost 100 years, and an extensive wealth of literature has accumulated on this subject. Nitrosyl halide reacts with most of the elements and with an extremely wide range of compounds. Comprehensive literature reviews[1-3] have summarized the present state of knowledge and it is necessary here to highlight some of the salient features of the nitrosyl chloride and nitrosyl bromide (NOCl and NOBr) reactions upon organic compounds including nitrosyl chloride reactions upon fatty acids.

Reactions of Nitrosyl Chloride with Carbon-to-carbon Multiple Bonds

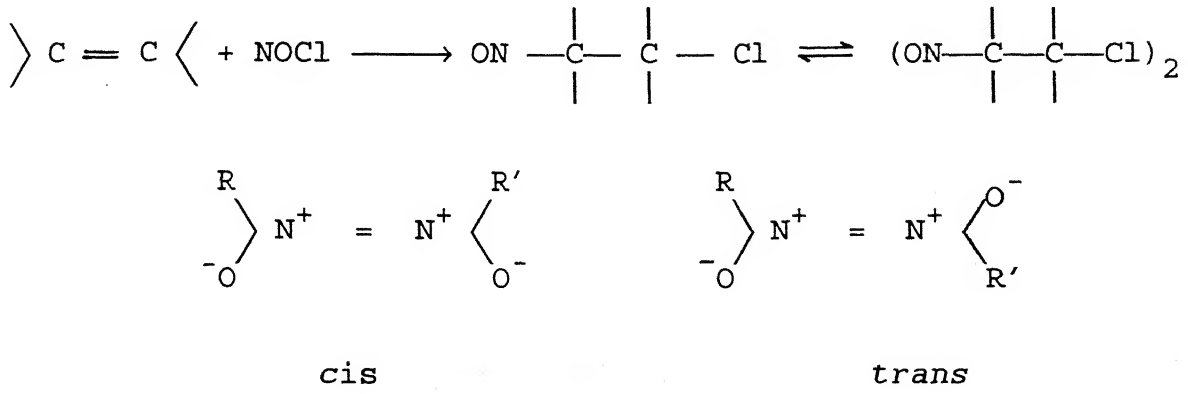
The reactions of this classification involve

principally the addition of nitrosyl chloride to double bonds, i.e. nitrosochlorination. During the present century less emphasis has been placed on reactions with terpenes and more study has been devoted to the application of nitrosyl chloride in the treatment of complex natural products and to its use in the synthesis of detergent and other materials.

Olefins add on nitrosyl halide where the halide atom being the negative and of the dipole in NO will add onto the carbon atom joined to the least number of hydrogen atoms (Markownikoff's Rule).

The reaction of olefins with NOCl to give nitrosochloride with dimerize if unhindered (eq. 1) has been since long[4], and has played an important role in early studies of terpenes. The use of an alkylnitrite and hydrochloric acid, generating the nitrosyl chloride *in situ*, provides a convenient, alternate technique for carrying out these additions. Nitroso compounds are usually blue or green liquids which dimerize to white crystalline solids often in equilibrium with the monomeric form. These bimolecular solids regenerate the monomer when fused or when dissolved in solution. Chiton *et al.* (1955) and Gowenlock *et.al.* (1995)[5] have found that these dimers have *cis* or *trans* configuration. Aliphatic *trans* dimers exhibit infrared absorption in the region 1290-1176 cm^{-1} where as

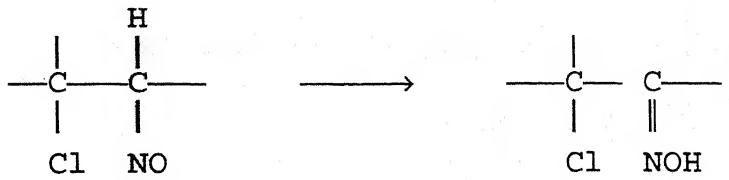
cis dimers have absorption in the region 1420-1330 and 1344-1323 cm^{-1}[6]. Monomeric C-nitroso compounds absorb in the region of 1498-1620 cm^{-1}[7].



cis *trans*

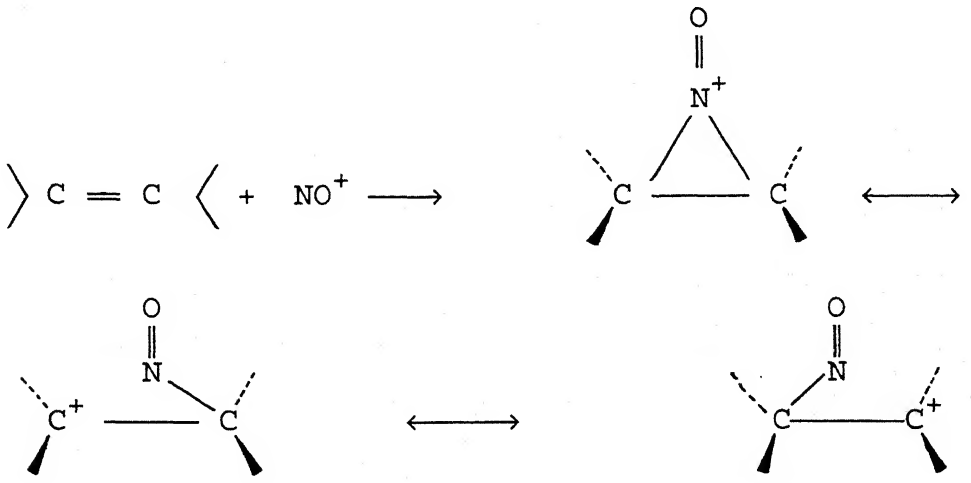
(eq. 1)

Isomerisation to oximido structures yielding chlorooximes is feasible where the labile hydrogen on the carbon of nitroso group attachment is available (eq. 2). The oximes are more stable since bonds between hetroatoms are always weak, and the oxime has only one such bond while nitroso has two.



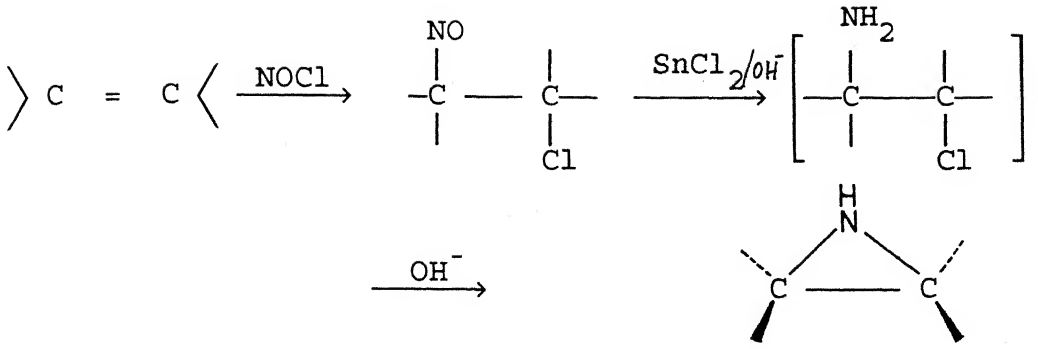
(eq. 2)

Mechanism involving NO^+ and Cl^- has been generally assumed for nitrosyl chloride addition to double bond[7-8]. For example, Kaplan, Kwart, and Schleyer[7] have suggested that nitrosyl chloride ionize to give the nitrosonium ion (NO^+) which adds to an olefin to give a highly stabilized onium ion intermediate (eq. 3), which should open to give a *trans* nitrosochloride.



(eq. 3)

A novel preparation of aziridines from tetrasubstituted olefins[9], consisting of nitrosyl chloride addition followed by stannous chloride reduction (to give a chloroamine) and base cyclization (eq. 4) is consistent with this proposal.



(eq. 4)

Since a knowledge of the stereochemistry of nitrosyl halide addition increase the synthetic importance of these reactions as well as help in elucidating their mechanism, Meinwald *et al.*[10] studied this problem explicitly for a variety of olefins. They found that the steric course of nitrosyl halide addition to olefins depends on the olefins structure. Thus Δ^9-octalin gives a *trans* adduct, in accord with the generally assumed ionic reaction mechanism, and it is probably that most other unstrained olefins behave similarly[11]. On the other hand, the addition of nitrosyl chloride and nitrosyl bromide to norborane, anti-7-methoxy-norborne and norbornadiene follows a *cis* stereochemical course and is accompanied by molecular rearrangement[12-14] suggesting that if these reactions are ionic once, very little electron demand is made on these olefins in the transition state. There is a close similarity between the pattern of reactivity uncovered in this work and that shown the oxymercurization reaction. Unstrained olefins undergo

trans addition *via* an electrophilic mechanism, but certain strained alkenes such as norbornene have been shown to give a *cis*-oxymercuration products[15]. Meinwald *et al.*[10] postulated a single mechanism to accommodate both cases. As a first step, the olefin would react with the nitrosyl halide to give an onium ion, as discussed above, with the cyclic contributing structures being the most important. For an intermediate in which *trans* displacement of one of the C—N bonds is sterically acceptable, the cyclic intermediate is opened by attack of halide ion to give, a *trans* product. For a more constrained substrate, in which such a *trans* displacement would require a difficult twisting about C—C bond in a relatively inflexible system, it may be postulated that attack of halide ion from a *cis* position is more favourable, and a *cis* adduct results.

A decision between those possibilities does not seem possible on thye basis of the data now available. It is hoped, however, that the demonstration of a relationship between the structure of an olefin and the streochemistry of its derived nitrosyl halide adduct may prove useful.

In contrast to the nitrosyl chloride addition reactions which take place with olefinic groups at low temperature, chlorination or oxidation effects are obtained at elevated temperatures and in some cases even at room

temperature. The reported formation of nitro derivatives from nitrosyl chloride and some chlorinated olefins[16] apparently involves oxidation of the initially formed nitroso compounds.

Addition of nitrosyl halide (NOCl and NoBr) to higher molecular weight olefins or derivatives and improvements in the procedures have formed the basis of a number of patents for the manufacture of surface-acting agents. Generally the nitrosyl chloride addition products of the high-molecular-weight olefins are found to be liquids.

Although addition of nitrosyl chloride to terpenes and the simple olefins has been widely studied, addition to unsaturated fatty acid derivatives has received little attention. It was shown in 1894 by Tilden and Forster[17] that nitrosyl chloride adds to oleic and elaidic acids quite readily but their isolation of solid products seems improbable in light of the work reported by Miller *et al.*[18].

A patent[19] has disclosed the preparation of surfactants from nitrosyl chloride adducts of oleic acid and its esters. Of particular interest is a paper by Kaufmann and Rover[20] reporting analytical method for unsaturated fatty materials based on addition of nitrosyl chloride in a

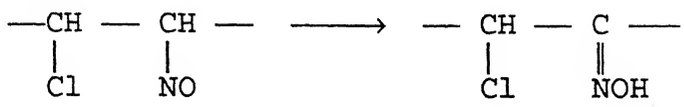
manner analogue to that used with iodine monochloride in the standard iodine value determination[21].

However only the disappearance of NOCl was measured, and no attempts were made to isolate products. They state that their studies would be directed toward preparative work based on nitrosyl chloride adducts of unsaturated fatty materials. Miller *et al.*[18] later on, reported the successful addition of nitrosyl chloride to methyl oleate on a preparative scale (at 2^{o} methylene chloride as solvent) and some reactions of the products. The product, methyl 9(10)-chloro-10(9)-nitrosostearate, is formed according to equation (5).

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOCH_3 + NOCl \longrightarrow CH_3(CH_2)_7\underset{(Cl)}{\underset{|}{CH}}\text{-}\underset{(NO)}{\underset{|}{CH}}(CH_2)_7COOCH_3$$

(eq. 5)

The dimeric product which is so often observed in the reactions of nitrosyl chloride with olefins is not formed in significant amounts under these conditions. The usually facile rearrangement of the secondary nitroso compound to the oxime (eq. 6) proceeds slowly on standing and is not easily accelerated.

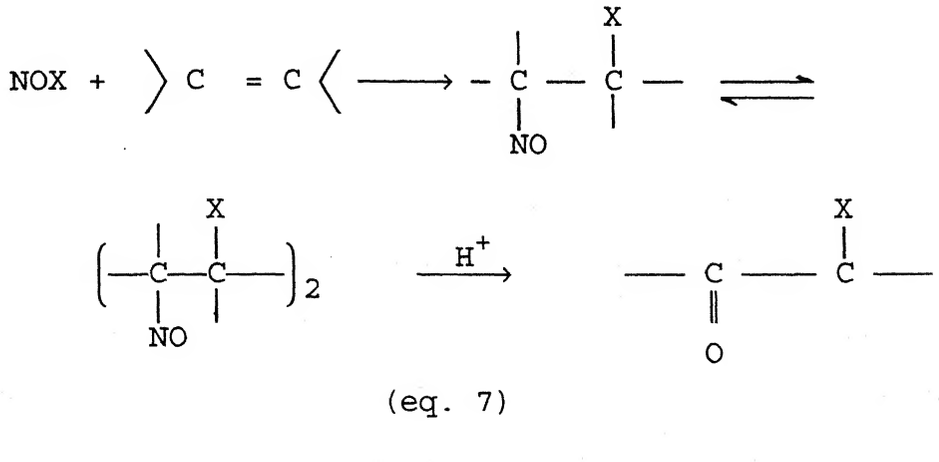


(eq. 6)

The stereochemical course of the nitrosochlorination of methyl oleate has not been investigated, and the literature concerning the nitrosochlorination of other olefins does not provide a satisfactory guide. Undoubtedly, the nitrosohalogenation of unsaturated fatty acids and other long-chain aliphatic olefins needs further investigation, since it is potentially useful in the synthesis of fatty nitrogen derivatives. A superficial examination of some of the reactions of methyl chloronitrosostearate has indicated that a variety of products can be prepared[18] and has shown the value of this type of adduct as an intermediate. Not yet demonstrated, but certainly within the realm of reasonable possibility, is the conversion of fatty chloronitroso derivatives, to the valuable aziridines (eq. 4) and nitroaziridines[33].

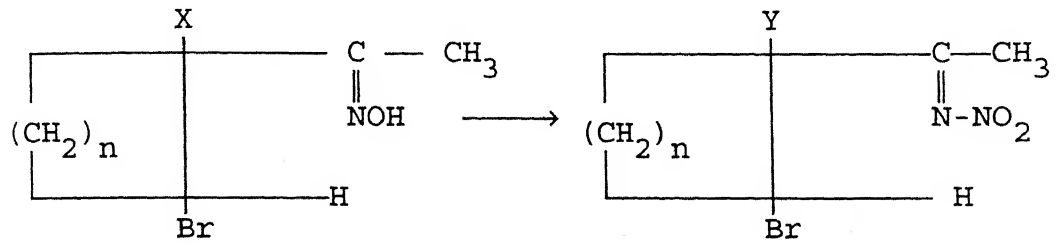
The addition reaction of nitrosyl chloride with olefins, with subsequent hydrolysis of the adduct with levulinic acid made 0.1 N in hydrochloric acid has been shown to be a convenient general method for converting olefins to the corresponding chloroketones[22] (eq. 7). Hydrolysis presumably proceeds *via* oxime tautomer of the monomeric nitroso compound. There is all possibilities for

converting olefinic fatty acids to the corresponding chloroketones by the application of this reaction.



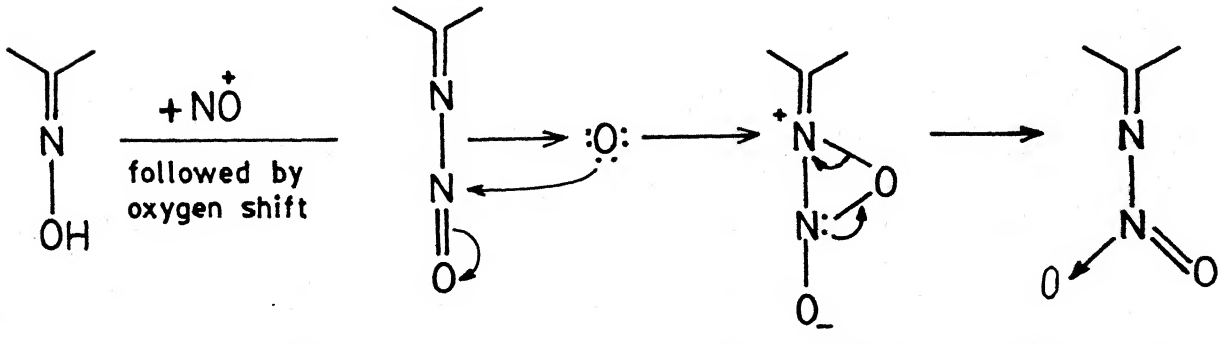
(eq. 7)

Normal addition of nitrosyl chloride to an olefin gives a chloronitroso product (monomer or dimer) or an α-chlorooxime. Other products have been called anomalous[2-3]. The normal (primary) products may be oxidized to secondary products. Shiue et. al.[23] have found a quite different result when compounds of type I are treated with NOCl. Two reactions occur. The oximino group is oxidized to a nitrimine (II) (eq. 8), a result that has been accomplished by nitrous acid oxidation[24,25] and by nitrosyl flouride[26] but not by NOCl. The oxidizing action of NOCl has been established.



(I) (eq. 8) (II)

The mechanism of nitrimine formation (new N-N bond formation at the oximino nitrogen by a electrophilic NO^+ group, followed by an oxygen shift) suggested by Freeman[24,25], and supported by Boswell[26] seems adequate to account for these results (eq. 9)



(eq. 9)

The sharp OH absorption at 3600 cm^{-1} characteristic of oximes[27] in dilute CCl_4 solution disappears as the reaction

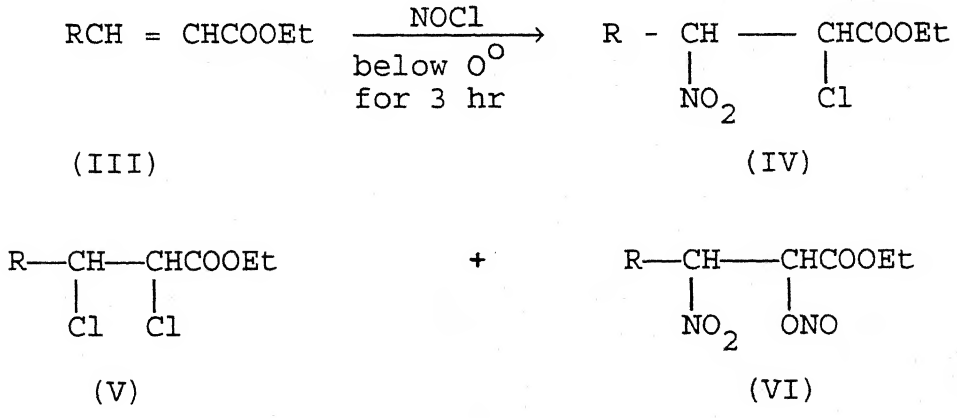
occurs. Compound II show strong bands at 1580 and 1320 cm^{-1} (NO_2) and medium bands at 1640 cm^{-1}(C==N), characteristic of nitrimines[28]. In the following years, 1971, Shiue et. al.[29] reported additions to two ethylidenecycloalkenes and concluded that the chloronitroso addition is the only primary reaction. After that three pathways may be followed: (1) dimerization of the nitroso group (long known), (2) oxidation of the nitroso group to a nitro group, and (3) isomerization to an oxime, followed by oxidation to nitrimine.

Three pathways compete. The second pathway appeared to be the only one in steroid example[30-31] where dimerization may be inhibited or very slow. Oxidation of an oxime to a nitrimine has been accomplished recently by nitrosyl chloride[23]. Isomerization of chloronitroso compound to the oxime is catalyzed by hydrogen chloride and goes very rapidly in polar solvents[29] so that dimerization and oxidation to a nitro group may not compete successfully in such solvents. The results reported by Shiue *et al.*[29] bear out Oglobine suggestion that stable dimer precipitation diminishes opportunity for oxidation to a nitro compound. Their work also suggests that rapid isomerization to oxime lowers nitro formation and increases nitrimine formation.

In the mass spectrometer, only one decomposition pattern is obtained from monomer, dimer and oximino form. So the dimer must dissociate and/or isomerize in the ion chamber. The maximum m/e observed is that of nitrosyl chloride monomer[30].

In 1972 Shin et. al.[32] carried out reaction of α,β-unsaturated carboxylic ester (III) with nitrosyl chloride and observed that the chief products are (IV) and (V) as shown in equation (10).

The reaction was carried by adding nitrosyl chloride in a stream to a solution of compound III in dry benzene cooled below 0^{o}, with an ice-salt bath. After being stirred for 3 hr at 0^{o}, this reaction mixture was allowed to attain room temperature and to stand for 5 days.



(eq. 10)

Recently the use of NOCl reaction on double bond has been made for the synthesis of N-nitroaziridine[33]. The reduction and subsequent base cyclization of nitrimine formed by the action of excess NOCl upon steroidal compound provides a route for the synthesis of N-nitroaziridine which were hitherto known as unstable compounds.

Reaction With Hydroxyl Group

In reactions with hydroxyl compounds, nitrosyl chloride functions as the acid chloride of nitrous acid, generally leading to the formation of nitrites. This reaction is identical in effect with the nitrosation of amino, methylene, and similar hydrogen-containing groups. In some cases, oxidation of the hydroxyl group take place, together with chlorination in other parts of the molecule.

In the gas phase reaction of methanol and nitrosyl chloride an equilibrium is instantly established even at 25° (eq. 11).

$$ROH + NOCl \longrightarrow RONO + HCl$$

(eq. 11)

In view of the equilibrium, it is readily understood why alkyl nitrite and hydrochloric acid formed a convenient means of preparing nitrosyl chloride *in situ* for organic reactions in most of the early work. Using dry

pyridine on an acid acceptor in liquid-phase reaction makes high yields of nitrites possible from alcohols such as amyl, n-octyl[35], d-3-nonanol[36] and tertiary alcohols such as 3-methyl-3-pentanol and 3-ethyl-3-hexanol[37]. Under conditions used successfully with the above alcohols, glycerol, ethylene glycol, chloretone, menthol, trimethylene chlorohydrin, and benzyl alcohol do not yield nitrites[37]. It is of note that d-2-octanol in reaction with nitrosyl chloride gives an 80% yield of the dextrorotatory nitrite[38].

Whereas hydroxyl groups attached to aromatic nuclei do not readily form nitrites.

Although much careful work has been done on the reactions of fatty acids, the search for purity and homogeneity was severely impleded by the lack of methods for determining the approach to this ideal state. Recent advances in chromatographic methods of separation and spectroscopic methods of structure determination make it possible that all the products of a reaction can now be examined profitably in fatty acids.

The use of spectroscopic methods has contributed a lot to our recognition of a variety of novel fatty acids and their derivatives and our understanding of their molecular structure and reactions. Out of the four spectroscopic disciplines, the use of NMR and mass in the study of fatty

acid identification and characterisation of their derivatives have attracted considerable attention in recent years. Therefore it is appropriate here to give a brief account of the application of NMR and mass spectrometry in the study of fatty acid chemistry.

Nuclear Magnetic Resonance (NMR) Spectroscopy

From the late fifties onward, applications of NMR spectroscopy have developed continuously, and they occupy a paramount position as a research tool in organic chemistry. The development of NMR has been characterised by a series of stages which have made it an increasingly powerful research technique and each of these has found an application to lipids. When the NMR spectrum of a simple molecule is determined, its chemical structure can often be elucidated by first order interpretation of spectral data. The number of different types of protons in the molecule can be determined by integration of peak area and information about proton environment can be obtained from the chemical shift, multiplicity, and coupling constants of distinguishable peaks.

In recent years various techniques have been developed to extend the application of NMR to compound of complex structure. Among these are: (a) addition of D_2O to

suppress the signals of -OH and $-NH_2$ protons, (b) determination of spectra in various solvents to obtain information from solvent effects, (c) application of decoupling (double resonance or double irradiation) to simplify complex signals and to identify related protons, (d) repeated scanning and averaging by computer to obtain definite spectra in very small samples, (e) the use of shift reagent in structural determination, and (f) ^{13}C NMR.

A number of reviews[39-42] on the NMR spectra of fatty acids have appeared in the literature. The first commercially available NMR instruments were mostly of the 60 MHz variety and were primarily for recording proton spectra. It was recognized that 60 MHz NMR spectra of fatty methyl esters, including those of PUFA, contained signals which corresponded to groups of protons in various environments along the hydrocarbon chain[43,44].

Introduction of 100 MHz instrumentation was the major advancement in NMR, and this was followed within a few years by 220 MHz spectrometers. Each of these refinements resulted in a considerable enhancement in the resolution obtainable with a corresponding simplification of spectra.

A comparison of 100 MHz ^1H NMR (PMR) spectrum of PUFA with those of 60 MHz spectrum reveals some sharpening of the various signals, especially the τ 7.75 triplet due to

protons α to the carboxyl group, and an apparent triplet centered at τ 8.3 associated with the methylene group which is β to the carboxyl group and also β to a double bond, where as in 220 Mhz NMR spectra, resolution of proton signals is enhanced to such a extent that each PUFA tends to give a distinctive spectrum[45,46]. It has been shown[46] that 220 MHz spectroscopy can be used to determine both the stereochemistry and position of double bonds, and the position of triple bonds, in the majority of fatty acids and esters.

Although NMR is used by lipid chemists, the technique is severely limited in scope and utility because in most long-chain compounds, the majority of chain methylene protons, for all practical purposes, are magnetically equivalent. High resolution NMR spectroscopy, a power full tool in many fields of organic chemistry, has been used to advantage in the study of some unsaturated fatty acids but has found limited application in the analysis of fatty acid derivatives due to coincident chemical shift of methylene protons. These protons yield a broad signal of overlapping resonances which preclude their identification and counting as well as the determination of their coupling constants. Since majority of the chain methylene protons are magnetically indistinguishable, it is impossible to confirm spectrally the presence or absence of

chain substituents or chain branching. Recently, some interpretive problems have been overcome by determining the spectra in the presence of chemical shift Reagents (CSR) which expand the NMR spectra of lipid derivatives, thus providing considerably more structural information than it has yet been possible to obtain. The best CSR developed so far are rare earth complexes of europium[47,48] or praseodymium[49], typical CSR complexes combine Eu (III) or Pr (III) with the anionic ligands: 2,2,6,6-tetramethyl-3,5-heptanedione or 1,1,1,2,2,3,3-heptafluoro-7, 7-dimethyl-4,6-octanedione; abbreviated designations for these complexes are Eu $(thd)_3$, Pr $(thd)_3$, EU $(Fod)_3$, and Pr $(Fod)_3$. CSR can markedly expand NMR spectra of compounds containing functional groups with lone pairs of electrons, if the lone pair can coordinate with the rare earth metal. The spectra are expanded because the chemical environment of protons near the coordination site is different from the environment of distant protons in the molecule. The signals of protons near the coordination site are therefore displaced. This displacement is directly related to the distance between the protons in question and the complexed metal atom; the smaller the distance, the greater the shift. Complexes containing Eu and Pr complement each other since, relative to tetramethylsilane (TMS), the Eu complexes shift proton signals downfield from their original position whereas Pr

complexes shift them upfield.

If a molecule contains a functional group having sufficient Lewis basicity, it can form a complex with chemical shift reagents. The bonding in CSR complexes is considered to be mainly, if not exclusively, dipolar[49] and it has been reported to decrease in strength as the Lewis basicity of the functional group decreases : amine > alcohol > ketone $\geq$ aldehyde $\geq$ ether > esters > nitriles; halide, indoles and double bonds are inactive[48]. CSR induce changes in the NMR chemical shift of proton signals because the magnetic environment of protons in complexed molecule differs from the magnetic environment of protons in an uncomplexed molecule. CSR complexation can often provide additional spectra data for protons upto eight carbons away from a CSR - active functional group. Sometimes, however, due to overlapping NMR signals, useful information can only be obtained for protons within five carbons of a Eu $(fod)_3$ co-ordination site[50].

Chemical shift reagents can substantially increase the amount of structural information obtainable from NMR studies of saturated and unsaturated lipid derivatives. It is theoretically possible to obtain more information from CSR studies of unsaturated lipid derivatives by introducing additional CSR-active functional groups into these molecules

through derivatisation of their double bonds. However, additional CSR coordination site complicate spectral interpretation, because they increase the number of signals that overlap. The two model compounds viz., methyl ricinoleate and methyl 12-hydroxystearate were investigated[51] to test the feasibility of attempting other CSR analyses of polyfunctional molecules of unknown structure. The individual proton signals have been observed and assigned for all the protons inmethyl recinoleate, except those on carbons, 5,6 and 7. Information obtained for all protons in methyl-12-hydroxystearate, although in some cases several proton signals overlap.

In 1972 Wineburg and Swern[51] have reported that a single spectrum CSR analysis of a polyfunctional molecule is not possible. Unambiguous assignment of overlapping protons signals can be accomplished only through the use of several complementary interpretive techniques including an incremental addition study, the construction of proton plots and the calculation of induced shift ratios.

Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Spectroscopy

In ^{1}H NMR (Proton Magnetic Resonance) spectroscopy, the investigator examines signals which are associated with hydrogen atoms and give information about

the environment of those hydrogen. In a variation of NMR spectroscopy developed more recently "Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR)" peaks due to carbon atoms are recorded instead. This relatively new form of NMR is already being applied to fatty acids, and promises to be a powerful technique[52,54].

In Carbon-13 NMR signals, the multiplicity is determined primarily by the number of protons attached to the carbon under consideration; those with no protons attached, e.g. in carbonyl groups, appear as singlets. In many ^{13}C-NMR spectra, a hopeless profusion of overlapping signals is displayed without application of a technique called proton decoupling; the procedure collapses all multiplets to singlets so that the spctrum is greatly simplified[55,56].

Tulloch *et al.*[57] have assigned chemical shifts to all the separate signals in the ^{13}C-NMR spectra of methyl stearate, oleate, and petroselinate by means of the second and third atom isotope effects in the spectra of specifically deuterated esters.

All the isomeric oxostearates and most of the hydroxy and acetoxystearates can be distinguished and identified by their ^{13}C spectra. Bus et. al.[58,59] have studied^{13}C NMR of methyl, methylene, and carbonyl carbon

atoms of methyl alkenoates and alkynoates, and double and triple bond carbon atoms of unsaturated fatty acid methyl esters. Gunstone *et al.*[60,61] have made ^{13}C NMR studies of acetylenic and olefinic fatty acids and esters. Smith, Jr.[62] has studied the ^{1}H-decoupled spectrum of a conjugated acetylenic polyunsaturated fatty acid methyl isanolate. Recently the carbon-13 pulse Fourier transform NMR technique for measurement of intact plant tissue has been used by Chen *et al.*[63] for the characterization and estimation of fatty acid composition in seeds of Avena fatua, *Leucas cephalotus, and Stocksia brahuica.*

Mass Spectrometry (MS)

In recent years mass spectrometry has been widely accepted as one of the most valuable and powerful techniques available to the organic chemist for the structure determination of an ever-increasing variety of natural products. Within these areas, fatty acid esters occupied a unique position in that they represent one of the earliest and most comprehensively studied classes of natural products to be investigated. The use of mass spectrometry for determining the structure of fatty acids has been reviewed by Mc Closkey[64], Zeman and Scharmann[65] and Klein[66].

The successful mass spectral analysis of glyceraldehydes and their derivatives was coincident with the introduction of direct insertion techniques leading to the analysis of triglyceride mixture[67,68]. Combined gas chromatography and mass spectrometry (GC-MS), associated with refinements in the design of various types of molecular spectra[69,70], has been applied to the analysis of mixtures of fatty acid esters[71,72].

Most recent studies of the mass spctra of highly polar lipids such as glycerophospholipids, sphingophospholipids and glycolipids have used a wide range of techniques including 'soft' ionization methods to limit the fragmentation of the molecular or quasi-molecular ion.

Apart from low resolution mass spectrometry (LRMS ≅ 1000) which is the sine qua non of the analytical approach, more specialized techniques include (i) high resolution mass spectrometry (HRMS : RP ≅ 10000) for the accurate measurement of ionic mass to charge ratio, (ii) specific labelling with stable isotopes, or with functional groups designed to direct fragmentation, (iii) reduction of the electron beam energy in order to limit fragmentation, (iv) metastable ion techniques for the elucidation of specific pathways, and (v) the measurements of ionic appearance potentials yielding thermochemical data.

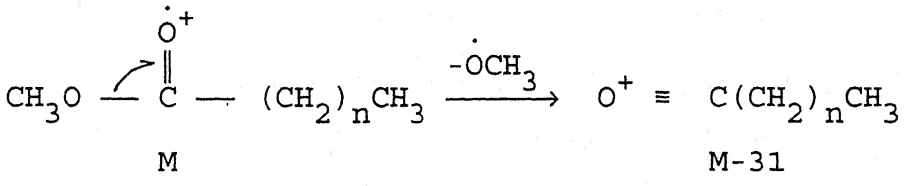
"Field desorption MS" technique developed by Beckey *et al.*[73] and Robertson *et al.*[74] has been used to obtain a greatly different mass spectrum consisting almost entirely of the molecular ion peak.

Mass Spectra of Fatty Acid Esters

Most of the mass spectrometric structure work on fatty acids has been performed on the corresponding (usually methyl) esters. Most fragmentation reactions can be classified as either simple cleavage or rearrangements.

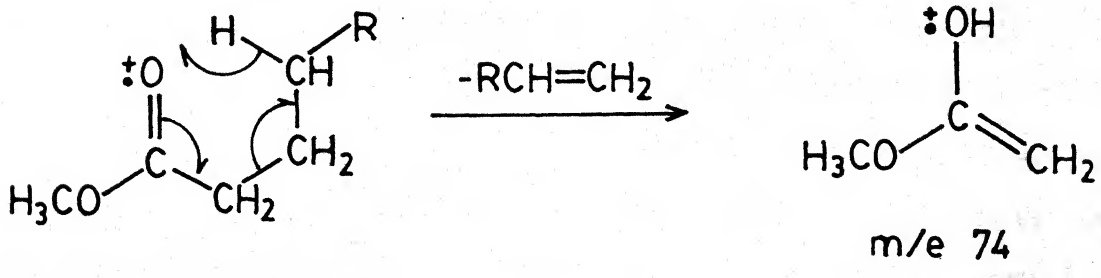
The Molecular Ion (M^+) and M-31

The relative abundance of M^+ increases from methyl pentanoate onwards and its presence can be verified by the acylium ion, M-31, formed by simple α-cleavage. This peak is of excellent diagnostic value of esters since it is characteristic of methoxyl group in methyl esters.



Mass-74

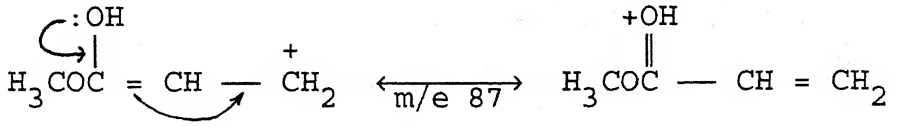
Gamma hydrogen migration to a double bond followed by beta cleavage yields the ion 74 (Mc Lafferty Rearrangement)[75] which is the base peak.



Mass 74 will shift to correspondingly higher masses if C(2) is substituted. Mass 75 is usually observed to be more abundant than required by the isotope peak of m/e 74. Most of the observed m/e 75 peak is due to protonated form of m/e 74. The origin of second transferred hydrogen is not known but is apparently abstracted randomly from the chain.

Oxygen-containing Ions $[(CH_2)_nCOOCH_3]^+$

These ions are arithmetically found at m/e (59+14n), i.e., m/e 87, 101, 115, 129, 143, 157 etc. The lowest potential member, m/e 73, is essentially absent, probably owing to the unfavourable location of a positive charge adjacent to a positively polarised carbonyl group. The most abundant member, m/e 87, derives its stability from the enol form.



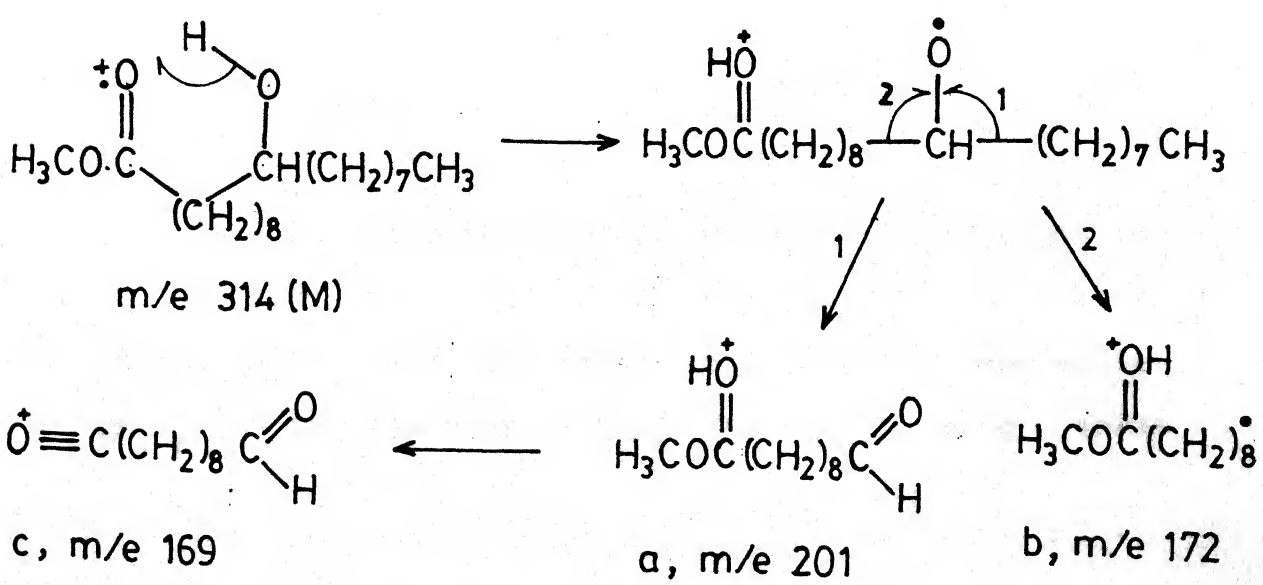
Hydrocarbon Ions

Both simple cleavage and rearrangement processes contribute to the formation of hydrocarbon ions, the most prominent of which (m/e 69, 83, 97 etc.) are from the saturated series C_nH_{2n+1}. The presence of hydrocarbon ions in the mass spectrum is general serves no structural purpose, but may occasionally be helpful in establishing a

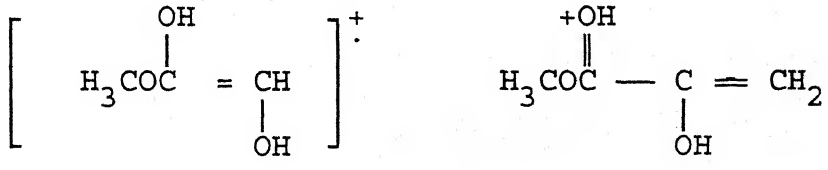
reference point for counting the spectrum.

Hydroxy Fatty Esters

In the mass spectra of hydroxy esters it has been observed that the molecular ion peak is usually small or absent. Taking an example of DL-10-hydroxy octadecanoate[76] the peaks at m/e M-32 (loss of methanol) and peaks at m/e 264, 222 and 180 characteristic for methyl oleate indicate that some dehydration has occurred. The location of the hydroxyl group is indicated by the base peak at m/e 201, the ion arising through α-cleavage on the far side of the hydroxyl group. Further loss of methanol from this ion gives the 'Ketone'-type ion of m/e 169. Another characteristic feature is the ion of m/e 172 that arises from α-cleavage in the near side of the hydroxyl group with shift of one hydrogen atom from the fragment lost.



Variation of the position of a functional group in the chain can give rise to considerable change in ion abundances, especially when the functional group is moved near either extreme end. For example, the mass spctra of methyl esters containing a hydroxyl group in position 3 are so dominated by m/e 103 (due to the α-cleavage on the far side of the hydroxyl group) that M and upper mass range fragment ions are virtually absent[76,77]. If the hydroxyl group is located on the alpha carbon atom, cleavage between C(1) and C(2) is facilitated, leading to loss of the carbomethoxy group. Ions of m/e 90 and 103 correspond, respectively, to the rearranged ion of m/e 74 and the ion of m/e 87 found for saturated methyl esters, the hydroxyl group being retained in the ion.



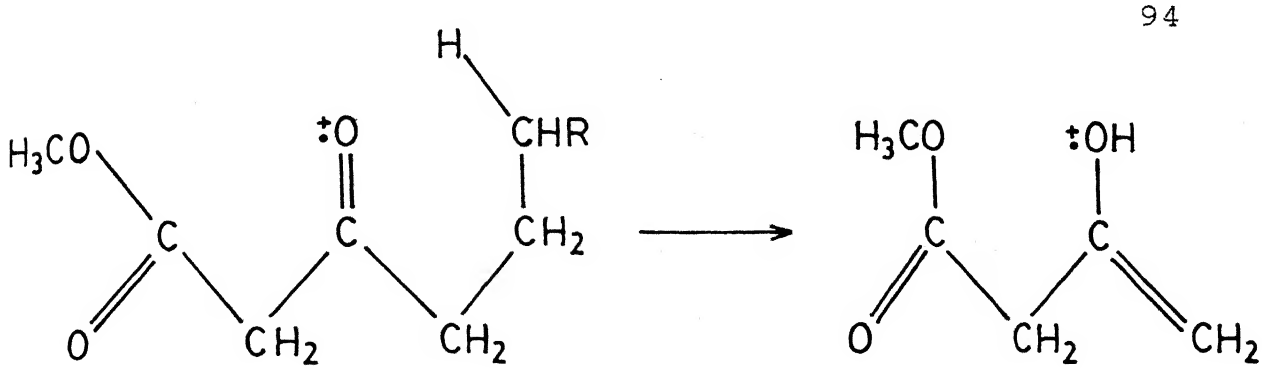
m/e 90 m/e 103

If the hydroxyl group is silylated, cleavages on either side of the substituted carbon atom result in prominent ions. For TMS ethers of diols in the series $CH_3(CH_2)_n$-CH(OTMS)-CH(OTMS)-$(CH_2)_m$-COO CH_3, cleavage takes

place between the two OTMS groups producing two fragments, $[CH_3(CH_2)_n\text{-}CH(OTMS)]^+$ and $[\text{-}CH(OTMS)\text{-}(CH_2)_nCOOCH_3]^+$ with the positive charge retained almost equally on both fragments. Salylation of hydroxyl groups in methyl esters of unsaturated hydroxy acids provides compounds that give mass spectra which can be readily interpreted, whereas spectra of underivatized esters are extremely difficult to evaluate. The relationship of the double bond(s) to the trimethylsiloxy (TMS) group results in specific mass spectral patterns.

Keto Fatty Esters

The position of a keto oxygen generally can be deduced easily from the mass spectrum[76] where both alpha and beta cleavages with rearrangement occur. Deviations from the pattern occur when the keto group is located near the methoxycarbonyl group or near the terminal carbon atom of the hydrocarbon chain. In the 2-oxo compound the tendency to cleavage between the vicinal oxo groups is so strong that the acylium ion of m/e M-59 dominates the spectrum. In case of methyl 3-oxooctadecanoate the base peak at m/e 116 is owing to ions formed by 4,5-cleavage (β to the 3-oxo group) with migration of one hydrogen atom as follows.



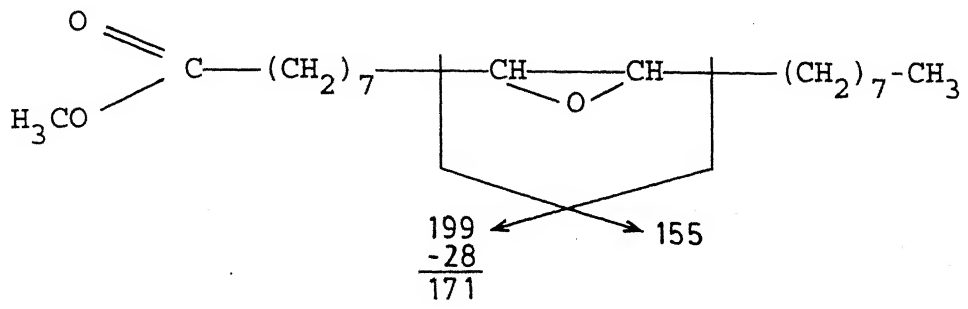
m/e 312 m/e 116

When the oxo group is located near the end of the hydrocarbon chain, the same cleavage pattern is observed except that for position (-2) and (-1) ions formed by β-cleavage on the hydrocarbon side of the oxo group and rearrangement are absent since no hydrogen atoms at γ-position with respect to the oxo group are available.

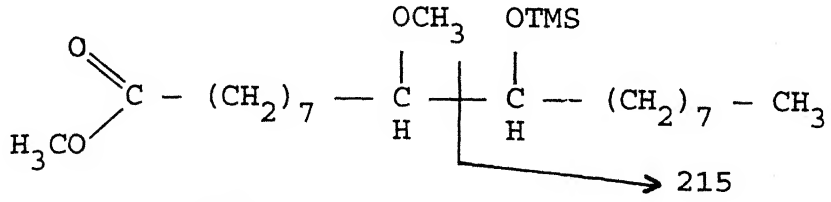
Epoxy Fatty Esters

Saturated epoxy esters give spectra, the interpretation of which is so straight forward that epoxidation and mass spectrometry form an established procedure to locate double bonds[78,79]. For example, the spectrum of methyl 9,10-epoxystearate[76] has a base peak of m/e 155 arising from cleavage alpha to the epoxide ring. Cleavage on the other side of the functional group produces a much smaller but significant peak at m/e 199. In contrast, addition of a double bond into the molecule changes the spectrum so radically that assigning the

location of the epoxide ring is almost impossible.



In 1972 Sen Gupta[80] converted the methyl esters of the epoxy acids into the cyclopentanone-ketals in the presence of BF_3 in traces, and studied their mass spectra to establish the structure of epoxy acids. Later on, Kleiman and Spencer[81] have studied the mass spectrum pattern by converting the epoxide to a methoxy-hydroxy group (by treatment with BF_3/MeOH) and then silylating. In esters without a double bond one methylene unit from the oxygenated site, two peaks define the location of the oxygenated group. Both peaks arise from cleavage between the carbon atoms containing the methoxy and the siloxyl groups. The significant ions contain the siloxyl groups and not the methoxyl groups. In esters that have a methylene group separating a double bond from the methoxy-trimethylsiloxyl groups, they also have the ions described above plus an ion from alpha-cleavage on the side of the methoxy-trimethyl-silyloxy substituent closest to the unsaturation.



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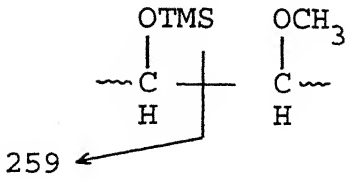

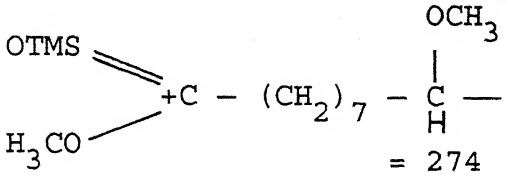

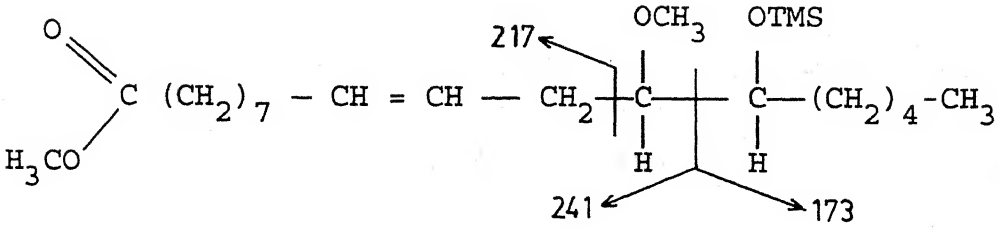

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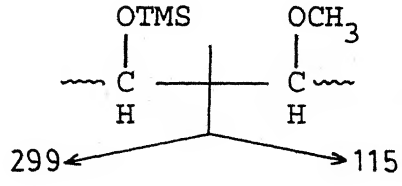


Unsaturated Fatty Acid Esters

The location of double bonds in fatty acids by ms has been studied in many ways which have been summarized in reviews[64-66,83]. Methyl esters of all positional and geometrical isomers of oleic acid (except the α,β-unsaturated) give mass spectra that are practically,

identical to that of methyl oleate[82]. The spectra of monosaturated esters are further indistinguishable from cyclopropane esters of the same number of carbon atoms. The problem of double bond location turns on the choice of a suitable derivative yielding distinct and limited fragmentation without migration, often directed by a charge-stabilizing group, such as TMS or a resonance-stabilized ring. Among these methods the procedure of methoxymercuration-demercuration has been reported[84] to be simple, reliable and rapid.

Mass spectra of methoxylated esters are characterized by intense peak due to cleavage adjacent to methoxy functions which allow the position of the original double bond in the chain to be ascertained. Fragments of the type $R-\overset{+}{C}H-\ddot{O}\,CH_3 \longleftrightarrow R - CH \equiv \overset{+}{O}\,CH_3$ are expected to be particularly prominent in the mass spectra of such methoxy esters. A modification of the method, which includes the mass spectrometry of methoxybromo/methoxyiodo derivatives of long-chain unsaturated esters prepared from methoxymercuric acetate adducts, has also been reported[85].

Anderson and coworkers[86,87] have reported a more satisfactory solution to the problem of determining the double bond position. These authors have demonstrated that more useful results are obtained with amides and

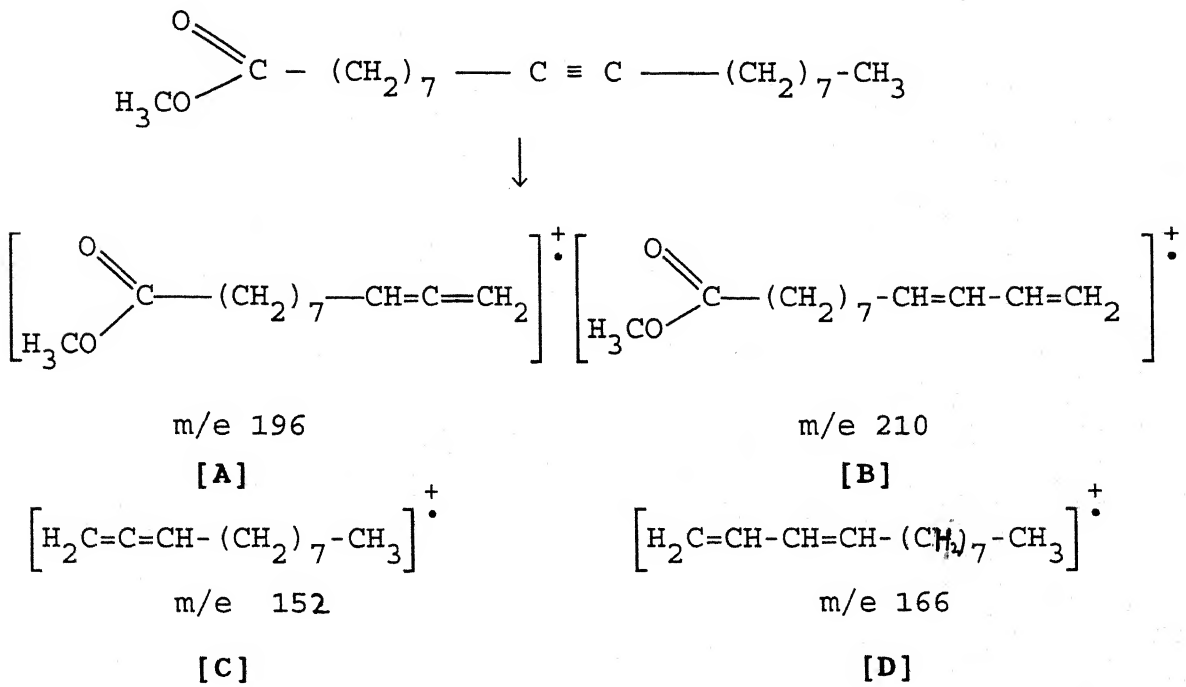
particularly pyrrolidides from the mass spectra of which double bond position can be deduced directly. The spectra of the octadecenylpyrrolidides contain clusters of peaks from the polar part of the molecule. If an interval of 12 atomic units, instead of the regular 14, is observed between the most intense peak of each cluster of fragments containing n and n-1 carbon atoms in the acid moiety then a double bond occurs between carbon atoms n and n+1 in the molecule. The rule is valid for the double bonds occurring at position C-5 to C-15 in an 18-carbon chain and has been applied to acids having 10-24 carbon atoms. Anderson *et al.*[88] have also applied the derivatization for mass spectrometric determination of double bond positions in polyunsaturated fatty acids (PUFA).

Plattner *et al.*[89] have developed a rapid micro-procedure to locate double bonds in polyenoic fatty esters containing one to four double bonds through partial oxymercuration.

Acetylenic Fatty Acid Methyl Esters

In 1976 Kleiman *et al.*[90] analysed an almost complete series of methyl octadecynoate (all but the 3,4 and 16,17 isomers) by mass spectrometry. The basic mass spectral pattern in one of cleavage with Mc Lafferty

rearangement either of the acetylinic bond or of the isomeric allenes found by rearrangement. For example, the mass spectrum of methyloctadec-9-ynoate (methyl stearolate) showed the four characteristic fragment ions:



m/e 196

[A]

m/e 210

[B]

m/e 152

[C]

m/e 166

[D]

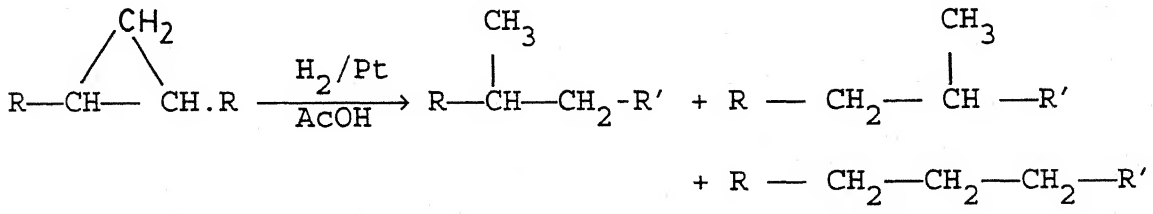
Ions with 32 mass units (CH_3OH) less than ions A and B were also present. Ions containing the terminal part of the molecule (C and D) are found most abundant when the triple bond is close to this part of the molecule. Fragment ions A and A-32 are the most intense of the characteristic ions when the acetylenic bond is near the ester function.

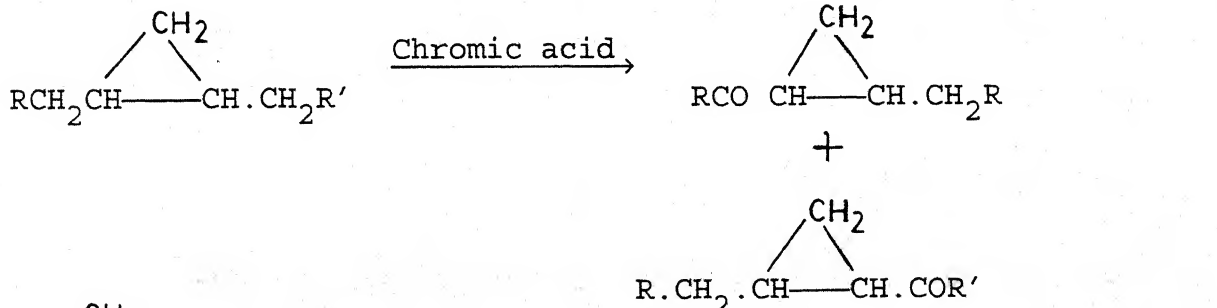
Oxymercuration of acetylenic esters produce both isomeric oxoesters, while addition of excess of $NaBH_4$ resulted in a mixture of hydroxy esters. The major ions

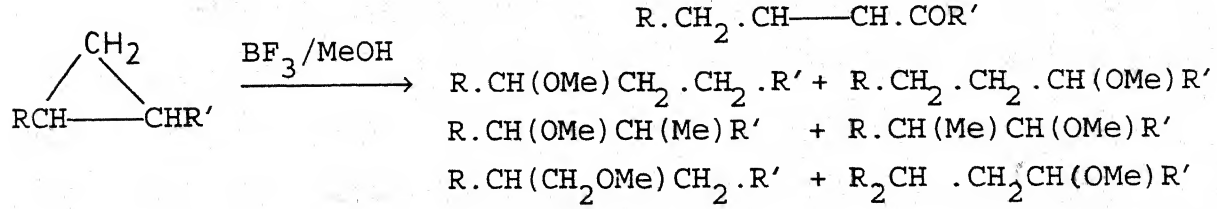
were determined for the silylated hydroxy esters formed from each member of the series. Each derivatized acetylenic ester produces upto four major and two minor characteristic peaks.

Cyclopropane And Cyclopropene Fatty Acid Esters

Cyclopropane fatty acids give mass spectra that are practically indistinguishable from those of the corresponding unsaturated isomers. The cyclopropane system may be fixed by some chemical reactions leading to a product, or more usually a mixture of products, which are identified by mass spectrometry. These reactions include hydrogenolysis[91], oxidation[92] and reaction with methanolic borontrifluoride[93].



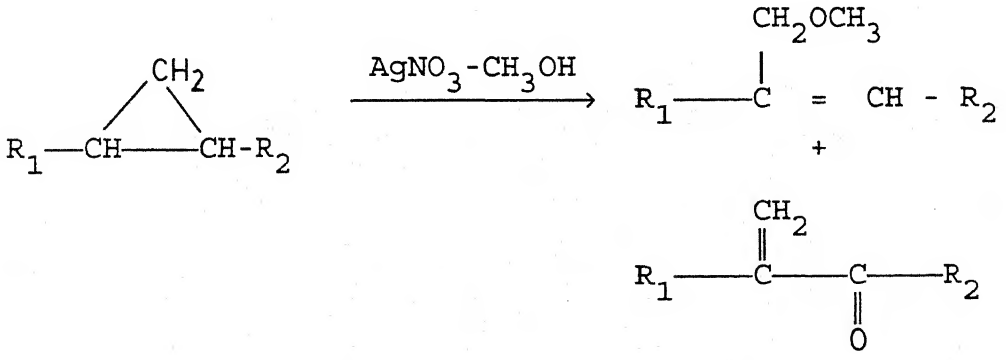




Gensler and Marshall[94] have reported the structure determination of cyclopropane-substituted acids by mass spectrometry. Chromium oxidation of cyclopropane fatty esters converts the alkyl methylene group next to the three-membered ring to an oxo group.

Cyclopropene esters are apparently too labile to be subjected directly to mass spectrometry, but the position of ring can be located by this technique if the compound is oxidized to a β-diketone[95] or reacted with methanethiol to form a product with the sulphur atom attached to either of the ring carbons[96].

Eicsele *et al.*[97] have studied the mass spectral fragmentation pattern of the silver nitrate-methanol treated derivatives of a number of cyclopropenoid compounds.



All the cyclopropene silver nitrate derivatives show a base peak of m/e 85, probably structure $C_6H_{13}^+$ or $C_5H_9O^+$. Ions at m/e 41, 43, 55, 71, 81 and 95 are intense in all spectra. Also, each methoxy derivative shows an ion equivalent to the loss of R_1 from the parent ion. A

characteristic parent minus 32 mass, which would indicate the probable loss of methanol from the parent ion, is present in all spectra.

Other large ring cyclic fatty acids such as those with cyclopentene[98], cyclohexene[99] or furanoid[100] give quite distinct spectra from which the structures are readily deduced.

B♦ PRESENT WORK

Although addition of NOCl to olefinic substrates[2,3,30,31] and nitrochlorination of unsaturated fatty acids and their derivatives have been carried out but no work has been done upon the nitrosobromination of unsaturated fatty acids and their derivatives. The reported addition of nitrosyl bromide upon olefins and norborene has highlighted the application of this reaction to fatty acid chemistry. The preparation of a variety of new fatty acid derivatives from internal, terminal and α,β-unsaturated acid are valuable contributions by S.M. Osman *et al.*[101-106]. As a part of our study of the derivatization of aliphatic compounds related to fats, the nitrobromination of olefinic fatty acids and their derivatives was taken up for the present study.

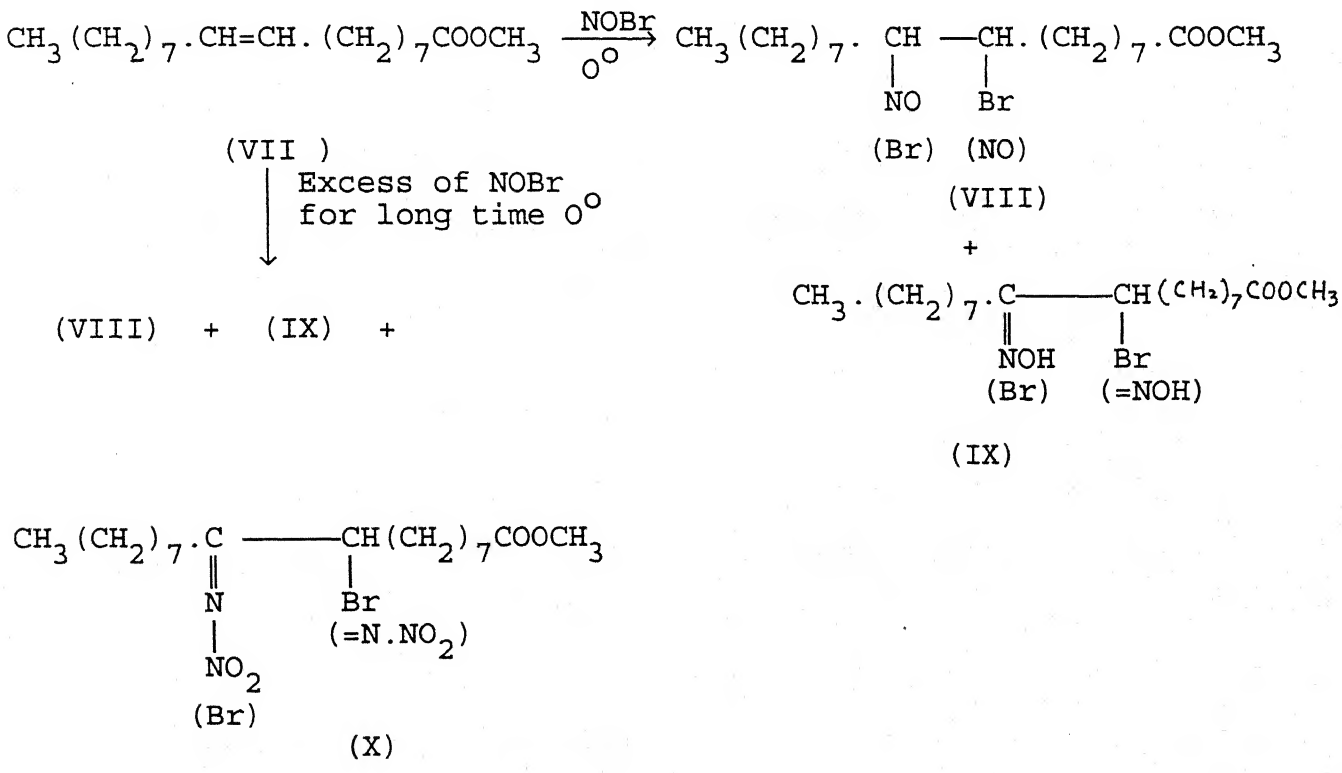
Nitrosobromination of Methyl Oleate (VII)

The nitrosochlorination of oleic acid was first carried out by Tilden et.al. in 1894[17]. Re-examination by Miller *et al.*[18] and Hasan *et al.*[107] has demonstrated that the addition of NOCl to methyl oleate is essentially quantitative. In continuation to this work the nitrosobromination of methyl oleate was carried out in the present investigation. The various products formed during the reaction of NOBr upon methyl oleate (Scheme I) under varying conditions were isolated and characterized with the

aid of chromatographic and spectroscopic techniques.

Methyl oleate (VII) on treatment with approximately stoichiometric quantities of nitrosyl bromide *in situ* (isoamyl nitrite + HBr) at 0^{o} in ethanol for 3-4 hr gave essentially quantitative yield of (VIII), in admixture with a little of its isomeric oximino form (IX). After usual workup of the reaction mixture, there was obtained a green liquid.

Scheme 1



Reaction products were separated into two fractions (1 and 2) by column chromatography over silica gel. A major fraction 1, eluted first as a green colour liquid contained chiefly a nitrosyl bromide adduct (VIII) is

admixture with a little oximino form (IX). With respect to the positions of the nitroso and the bromine, product (VIII) is probable a mixture of isomers [methyl-9(10)-bromo-10(9)-nitrosostearate, (VIII)]. That both isomers were indeed formed was evident from the TLC of the reaction product which showed two closely associated spots (R_f 0.8 and 0.85).

A minor fraction 2, of lower (R_f 0.23), was characterized as the oximino form (IX) of compound (VIII). This fraction was also believed to be an isomeric mixture of oximes [methyl-9-(10)-bromo-10(9)-oximinostearate, (IX)].The characterization of different fractions was made on the basis of microanalysis, IR and NMR.

Characterization of Fraction 1

The product was tested quantitatively for the presence of halogen by the Beilstein Test. It gave satisfactory microchemical analysis for $C_{19}H_{36}NO_3Br$ (compound VIII/IX). The IR spectrum (Fig. 1, sheet I) of fraction 1 showed, besides the bands usually found in long chain fatty esters, absorption at 1570 (N=0), 1110 (C-N) and 540 (C-Br) cm^{-1} indicative of the nitrosobromide functions. The presence of weak bands at 1640 (C=N), and 3440 (OH) cm^{-1} indicated the presence of ketoxime, a rearranged product of (VIII) in minor amount.

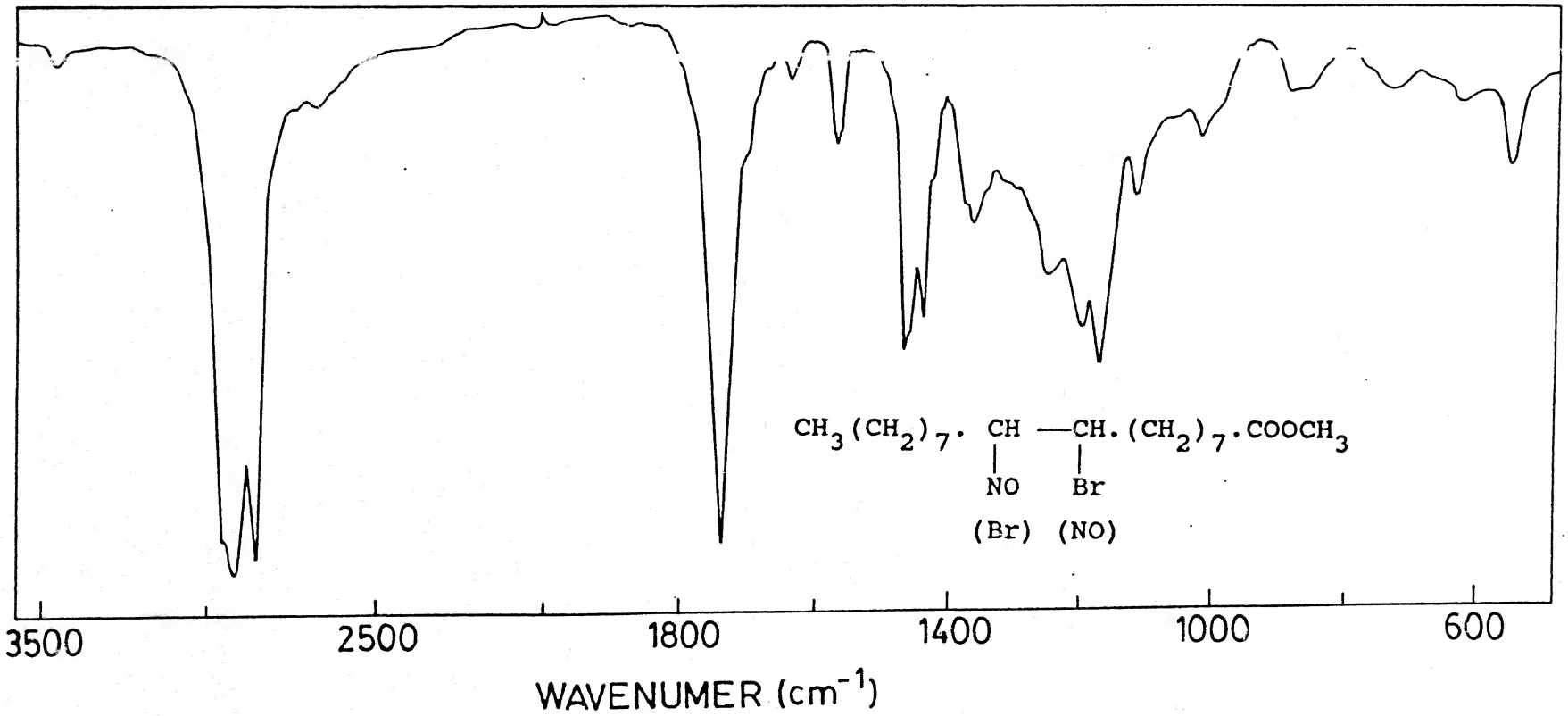


Fig.1. IR spectrum of methyl 9(10)-bromo-10(9)-nitrosooctadecanoate (VIII)

The NMR spectrum (Fig.2, sheet II) was decisive in arriving at a more firm conclusion regarding the composition of fraction 1 as an isomeric mixture of methyl 9(10)-bromo-10(9)nitrosostearate (VIII) and methyl 9-(10)-bromo-10-(9)-oximinostearate (IX), former being in a major amount. NMR spectrum displayed a signal at τ 2.52 (D_2O exchangeable) ascribed to the oximino group proton (=N-OH), an unresolved multiplet at τ 6.14 for methine proton adjacent to the bromine atom and an unresolved multiplet centred at τ 6.65 assigned to the methine proton adjacent to the nitroso group. A signal at τ 8.40 was also observed due to methylene protons α to the -CHBr- (-CH.Br-CH_2-]. Other proton signals signals usually present in fatty acid ester at τ 6.34 (s, 3H, $-\overset{\overset{O}{\|}}{C}.OCH_3$), 7.76 (2H, protons α to the ester group), 8.65 (br s, chain methylene protons), and 9.12 (distorted t, 3H, terminal methyl protons) were also observed.

Characterization of Fraction 2

Microanalysis of Fraction 2 (compound IX) supported the formula $C_{19}H_{36}NO_3Br$ (positive Beilstein test). IR spectrum of the compound (IX) displayed band at 3450 (OH), and 1640 (C=N) cm^{-1} indicative of the oximino group. NMR spectrum (Fig. 3, sheet III) showed an apparent multiplet centred at τ 2.65 for one proton which is D_2O

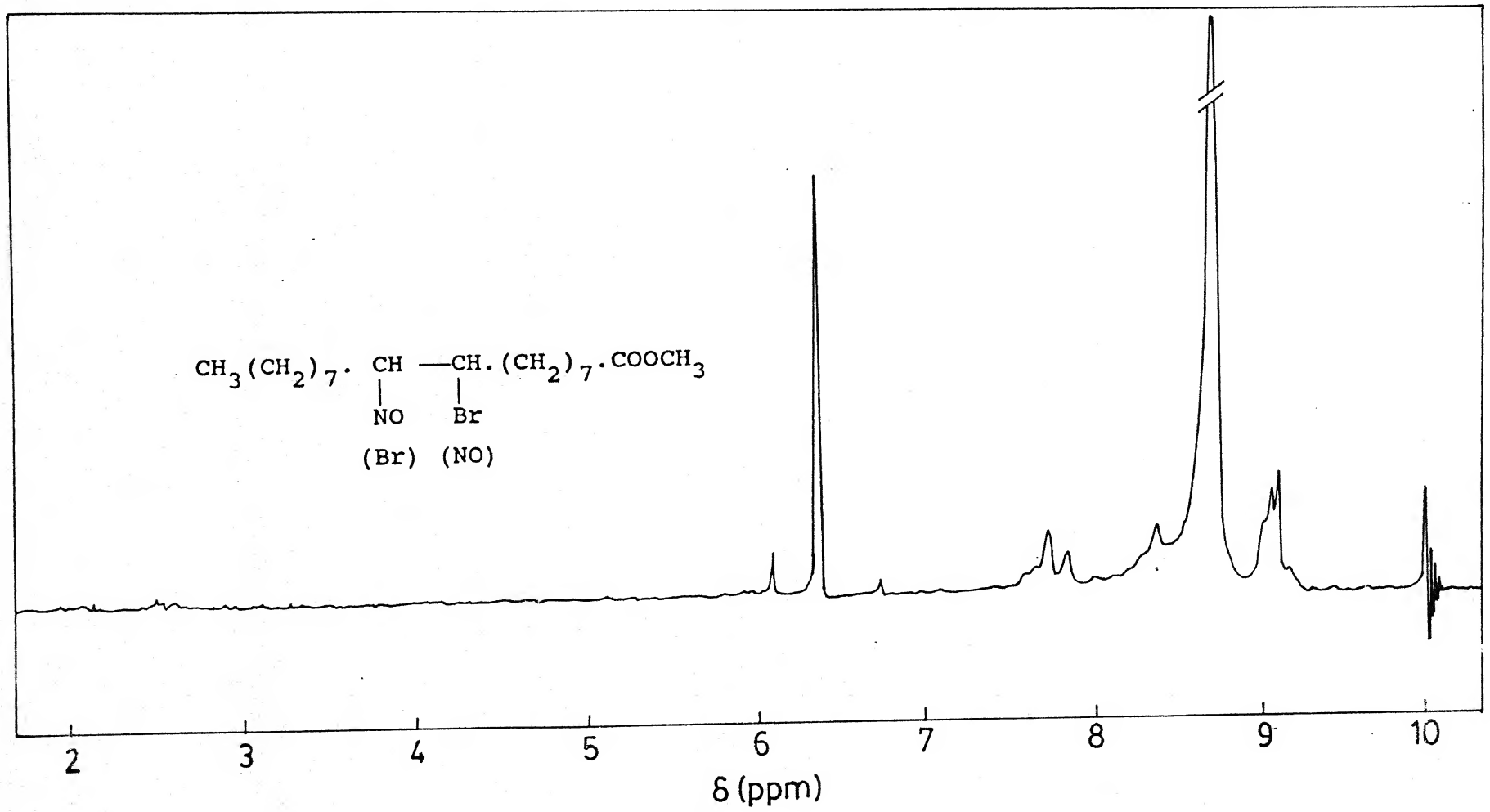


Fig.2. NMR spectrum of compound (VIII)

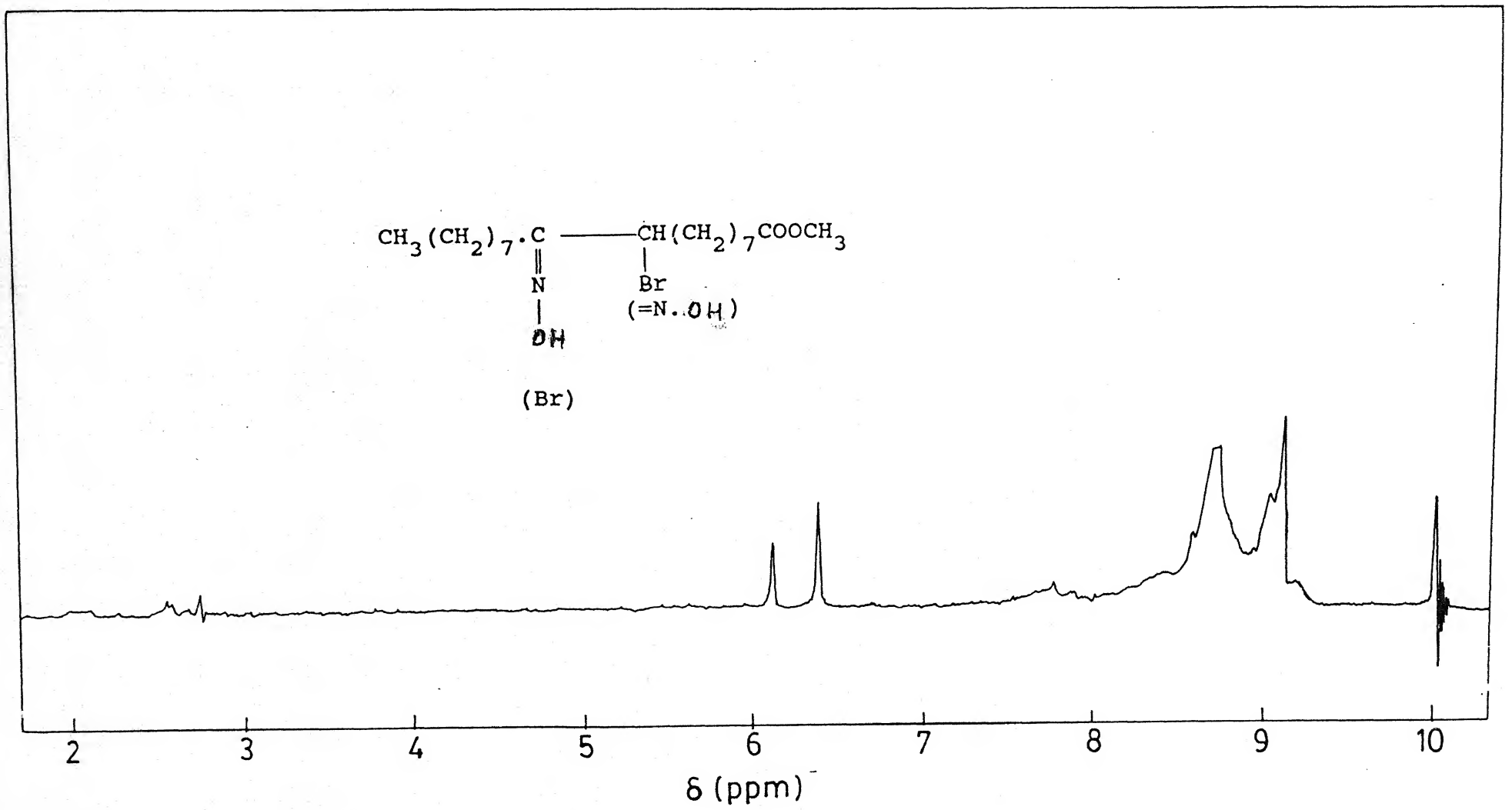


Fig. 3 NMR spectrum of methyl 9(10)-bromo-10(9)-oximinooctadecanoate (IX)

exchangeable and assigned to the hydroxyl proton of oximino group (=N-OH). Another signal at τ 6.18 is attributed to the methine proton adjacent to the bromine atom (-C*H*Br-). Other signals characteristic for long-chain fatty esters were also exhibited : the esters methyl protons ($-\overset{O}{\overset{\|}{C}} - O.CH_3$) gave rise to a singlet at τ 6.35, protons α to the ester carbonyl gave rise to a triplet at τ 7.76, the methylene protons appeared as a large broad signal centered at τ 8.65. A distorted triplet appeared at τ 9.12 for the terminal methyl group. Both IR and NMR substantiated the assigned structure (IX).

Presence of hydrogen on the carbon carrying the nitrosyl group permits rearrangement to the oximes[2]. For most of the examples cited in the literature this rearrangement is spontaneous or is promted under the mildest conditions. The product bromooxime is usually a solid, more stable than the nitroso isomer. But in this case isomerization seems to be very slow. About 0.5 - 1.0% of oxime was already present in the freshly prepared compound (VIII) as evidenced by TLC, IR and NMR. Oxime content increased at room temperature to a maximum of about 20% after about two weeks. As regards the formation of oxime our observations conform to those reported by Miller et. al.[18].

Many nitrosochloro compounds dimerize to white solids[2]. We have found no evidence for appreciable dimerization. The persistent green colour at 0-5° and the presence of the strong IR nitrosyl band as well as nonformation of any solid adduct, all indicate little, if any, dimer formation. Millet et. al.[18] also did not report the formation of dimer during nitrosochlorination of methyl oleate. Precedents exist for the suggestion that dimer formation is inhibited due to steric hindrance.

By treating with an excess of NOBr for a long time methyl oleate gave a product (X, 10%) in addition to products (VIII) and (IX). The product (X), having R_f value greater than the R_f of the oxime (IX) was characterized as methyl 9(10)Bromo-10-(9)-nitriminostearate on the basis of microanalysis, IR and NMR.

Characterization of Product (X)

Strong IR band at 1550 and 1360 (NO_2) cm^{-1} and a medium band at 1640 (C=N) cm^{-1} characteristic of nitrimines were observed. NMR spectroscopy (Fig. 4, sheet IV) was useful in confirming the structure of product (X). In addition to expected signals for remainder of the molecule (τ 6.34, 7.76, 8.67, and 9.12), diagnostically useful signals were observed at τ 5.90 (mc) due to the methine

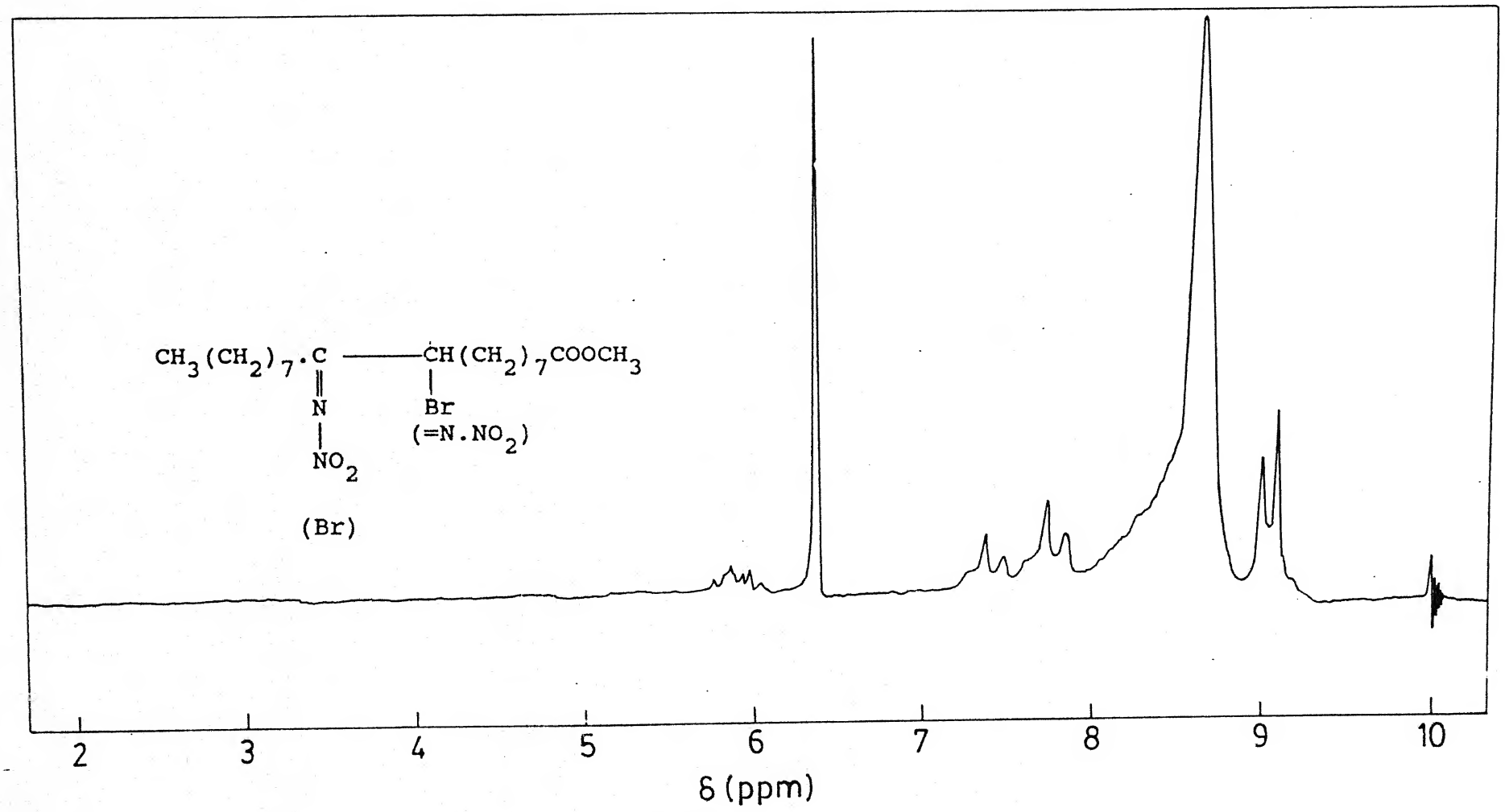


Fig.4. NMR spectrum of methyl 9(10)-bromo-10(9)-nitriminooctadecanoate (X)

proton adjacent to Br atom (-C*H*Br-) and at τ 7.38 (t) for methylene group α to the nitrimino group [-CH_2-C(=N.NO_2)-].

The nitrimine (X) is formed by the oxidizing action of NOBr upon oxime (IX). The oxidizing action of NOX to convert an oxime into nitrimine was first reported by Shive *et al.*[23] and later confirmed by other workers[29,33]. The mechanism of nitrimine formation (eq. 9) suggested by Freeman[24,25] and supported by Boswell[26] seems adequate to account for the results obtained in this reaction.

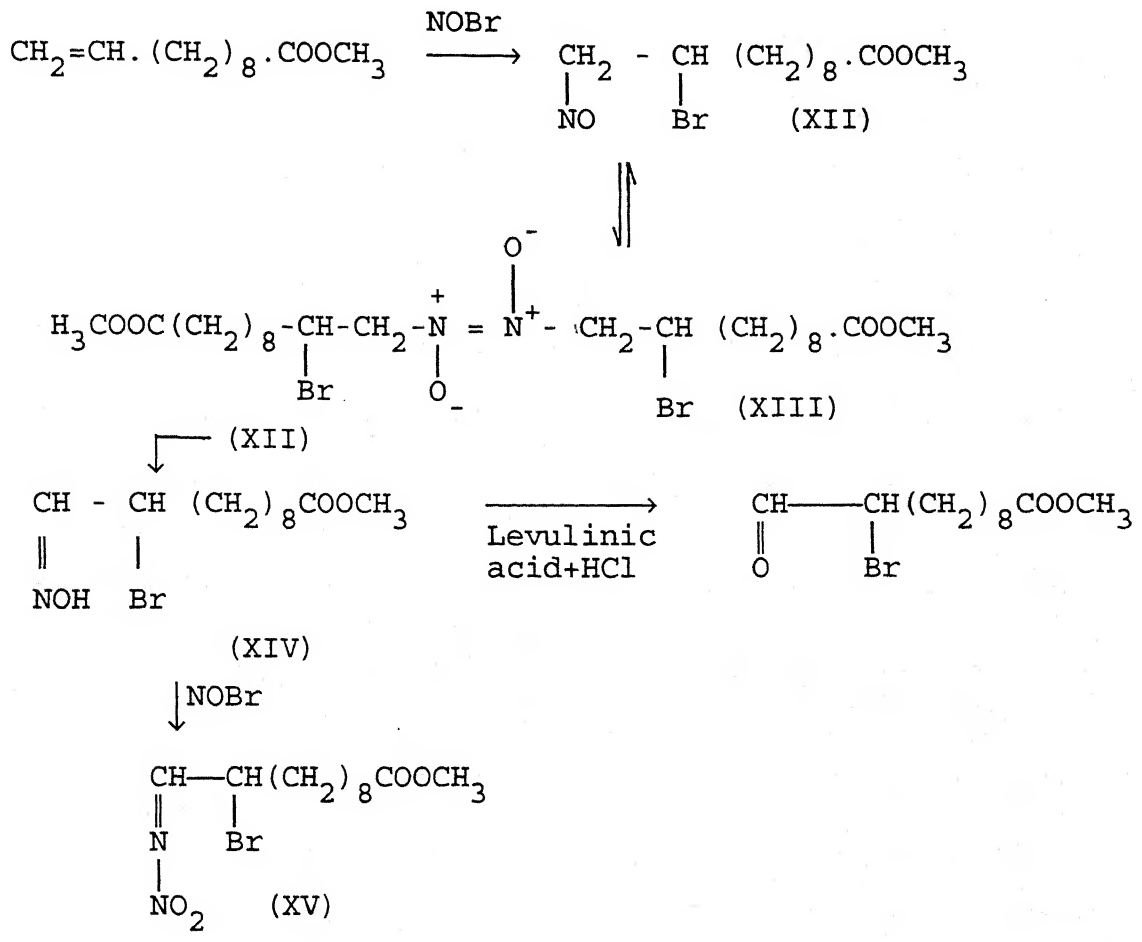
Although the formation of nitrimine was not reported by Miller *et al.*[18]. However, they osberved that when methylene chloride solution of compound VIII was treated with NOCl for a long time, IR showed the presence of nitro group in the product. From the foregoing results it is surmised that the nitro band is due to the formation of nitrimine which has been isolated and characterized in the present study.

Nitrosobromination of Methyl-10-Undecenoate (XI)

The study of the reactions of 10-undecenoic acid is interesting in fat chemistry due to a variety of reasons. The unique feature of 10-undecenoic acid is the presence of a terminal double bond. In order to probe the regioselectivity of nitrosyl bromide addition on an unsymmetrically substituted olefinic fatty acid, methyl ester of 10-undecenoic acid, XI, was selected as a model substrate for nitrosobromination reaction.

Reaction of methyl 10-undecenoate (XI) with NOBr *in situ*, resulted in the formation of four distinct products (XII-XV) as evidenced by analytical TLC. These components were separated by silica gel column chromatography. Formation of a bromonitroso product (XII) was indicated by the appearance of a bluish green colour in the reaction mixture. IR spectrum of the product also revealed the formation of nitrosyl bromide adduct. Work-up of the reaction mixture yielded no appreciable amount of the adduct in the pure form as it easily dimerizes or rearranges to an oxime.

Scheme 2



Characterization of the Compound (XIII)

The product (XIII) separated as white solid (m.p. 97°) gave satisfactory microanalysis for $(C_{12}H_{22}O_3NBr)_2$. The molecular mass determination by Rast method[108] in camphor supported the molecular formula $(C_{12}H_{22}O_3NBr)_2$ for compound (XIII). It gave positive Beilstein test. The IR spectrum (in nujol) showed, besides the bands usually found

in long-chain fatty esters absorption at 1270 cm^{-1} indicative of dimer formation (Fig. 5, sheet V). The absence of nitrosyl band in the region 1520-1570 cm^{-1} further supported the dimer formation. The NMR spectrum (Fig. 6, sheet VI) also supported the structure of compound (XIII) as dimer of methyl-10-bromo-11-nitrosoundecanoate. The NMR spectrum exhibited the significant signal at τ 5.46 for six protons due to the methine protons adjacent to bromine atom and methylene groups adjacent to nitrogen ($-CH_2-\overset{O^-}{\underset{|}{N^+}}=$). Other usual fatty ester signals were observed at τ 6.34 (s, 6H ester methyl), 7.76 (protons α to the ester $-\overset{O}{\overset{\|}{C}}-$ group) and 8.68 (br s, shielded chain methylenes). The dimer (XIII) appears to have a *trans* structure as suggested by Gowenlock and Luttke[6] in their IR spectral studies on dimers of nitroso compounds.

Characterization of the Compound (XIV)

The compound (XIV) was separated as a white solid (mp 45^{o}) in pure form having R_f 0.2. It responded to Beilstein test. The compound (XIV) was analyzed for $C_{12}H_{22}O_3NBr$. Its IR spectrum gave absorption at 3300 (OH) and 1680 (C=N) cm^{-1} attributed to the oximino group and at 540 cm^{-1} to C-Br linkage. Its NMR spectrum was more

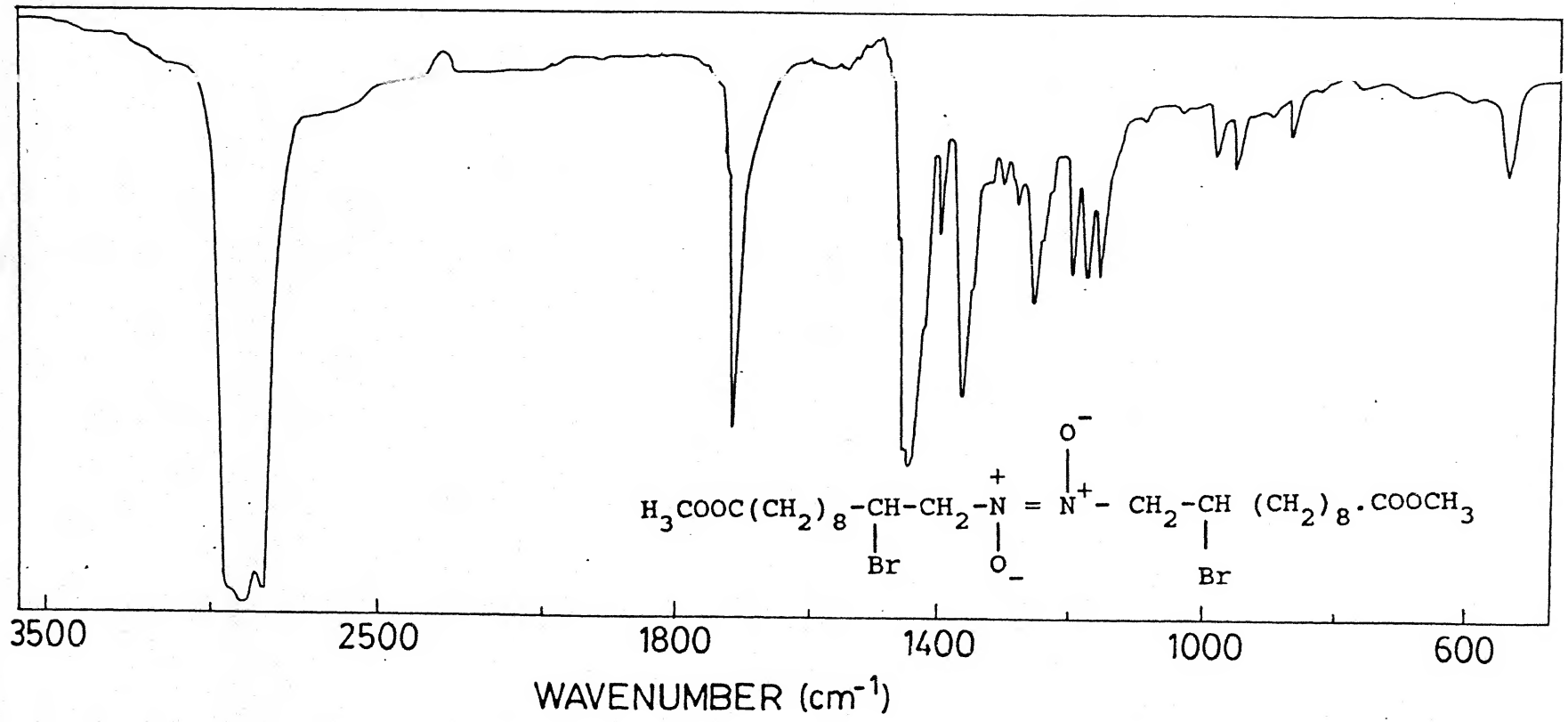


Fig.5. IR spectra of dimer of methyl 10-bromo-11-nitrosoundecanoate (XIII)

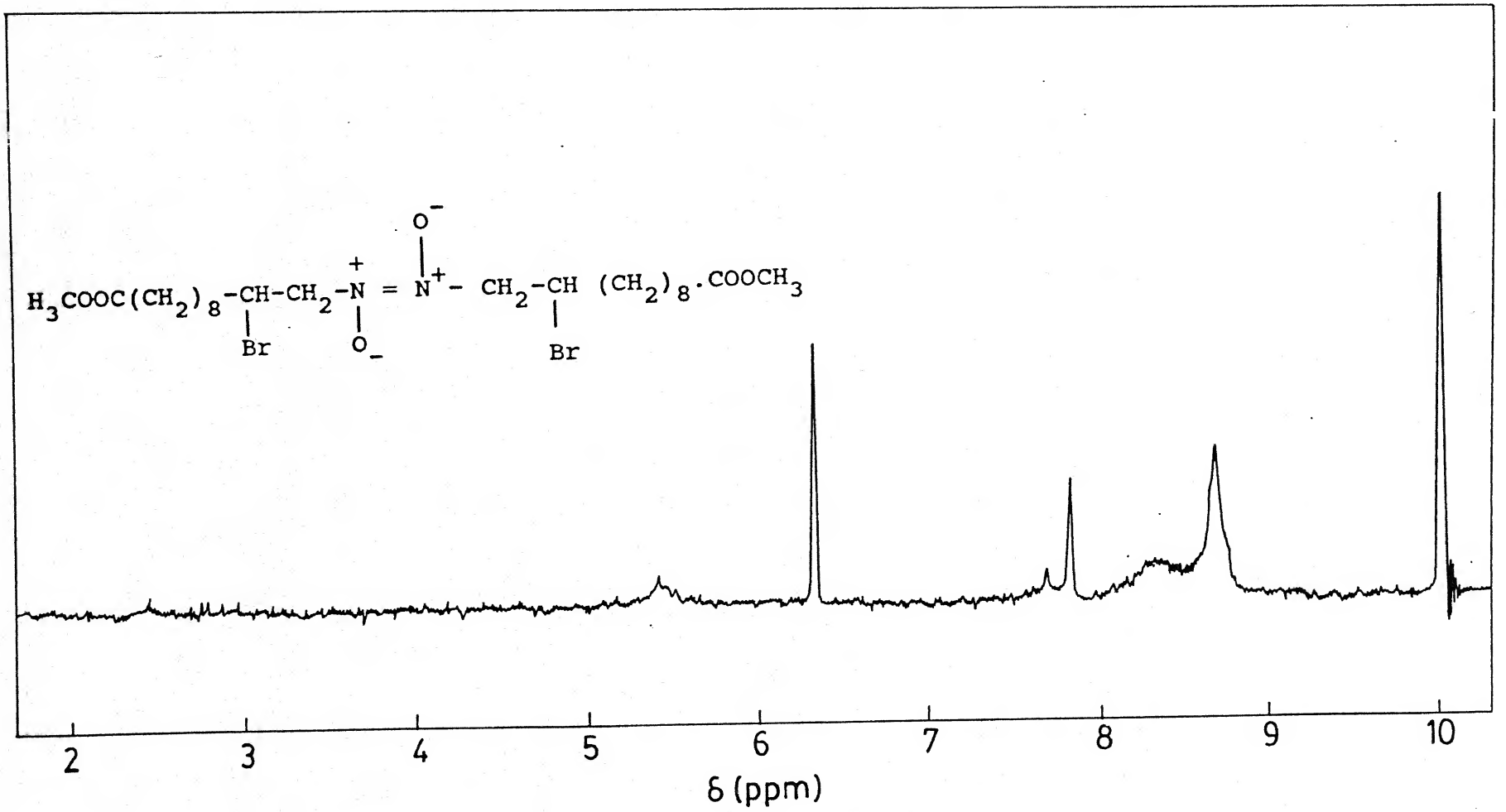


Fig.6. NMR spectrum of compound (XIII)

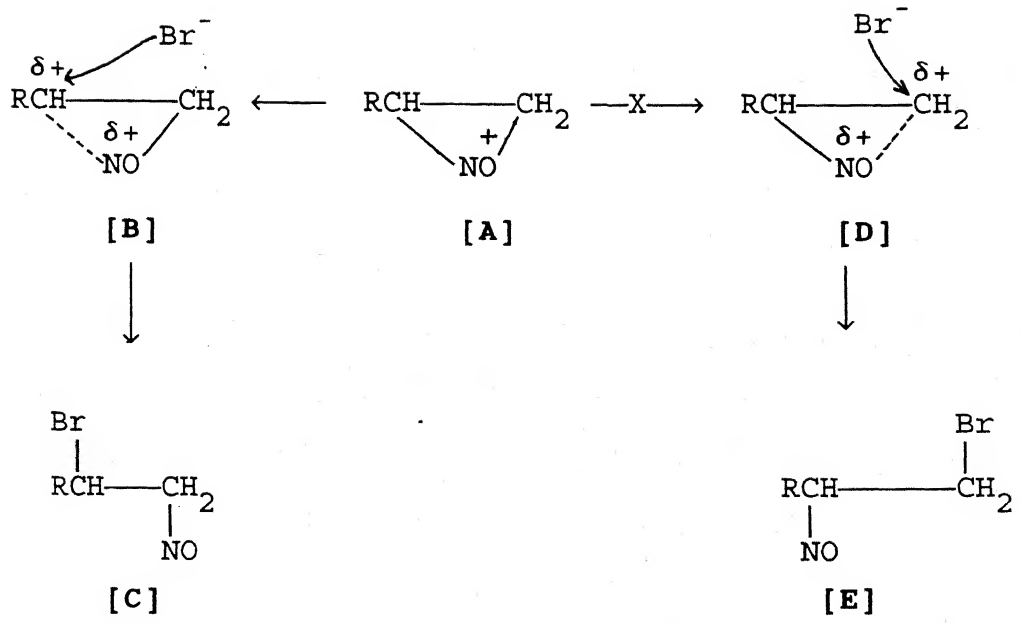
informative regarding the position of oximino group in the fatty acid chain. It exhibited an apparent singlet at τ 2.6 which can be assigned to the proton of oximino group (=N-O*H*). The proton was found to be exchangeable with deuterium. The proton was found to be exchangeable with deuterium. The proton at C-11 appeared at τ 3.6 (-C*H*=NOH), which conclusively proves the attachment of oximino group to the terminal carbon atom (C-11). Methine proton adjacent to bromine atom displayed a signal at τ 5.72. Other NMR signals were observed at τ 6.34 (s, 3H-$\overset{O}{\overset{\|}{C}}$-O$CH_3$), 7.76 (2H, α to the ester-$\overset{O}{\overset{\|}{C}}$- group), 8.65 (br s, chain methylene protons). Thus the spectral data established the structure as methyl 10-chloro-11-oximino-undecanoate (XIV). Further support to the structure was obtained from the analysis of the corresponding carbonyl compound (XVI) obtained by the deoximation of the product (XIV) with the help of levulinic and Hydrochloric acid[109]. The deoximation formed a compound (XVI) which was shown to have an aldehydic group. The presence of aldehydic group in product (XVI) was confirmed with the help of chemical test and spectroscopy. It gave yellow colour on heating with NaOH and reduces Fehling's solution. It also gave a positive DNP test on TLC. IR spectrum showed the disappearance of bands at 3300 and 1680 cm^{-1} (shown by oxime) and new bands appeared at 1710 (C=0)

and 2800 (aldehyde C-H str) cm^{-1} attributed to the aldehydic function.

Characterization of Compound (XV)

The compound (XV), which migrated ahead of oxime (XIV) on TLC plate, analyzed for $C_{12}H_{21}N_2O_4Br$ (positive Beilstein test). The IR spectrum showed bands at (C=N) and 1550 (NO_2) cm^{-1} characteristic of nitrimino group. NMR showed significant signals at τ 3.84 for one proton at C-11 (-C*H*=N.NO_2) and a multiplet centered at τ 5.46 for methine proton adjacent to bromine atom (-C*H*Br-). Other NMR signals usually displayed by the fatty acid esters (τ 6.34, 7.76 and 8.68) were also present. The product was thus assigned the structure as methyl 10-Bromo-11-nitrimino-undecanoate.

The formation of only one isomer (XII) in the nitrosobromination of 10-undecenoate indicated that the reaction is regiospecific and addition of NOBr is in accordance with the Markownikoff's rule. The exclusive formation of (XII) as primary product in the NOBr addition to methyl 10-undecenoate is consistent with the intermediary of a three membered ring ion, A, opening of which proceeds *via* the lower energy transition state (B rather than D where R can stabilized an incipient positive charge).

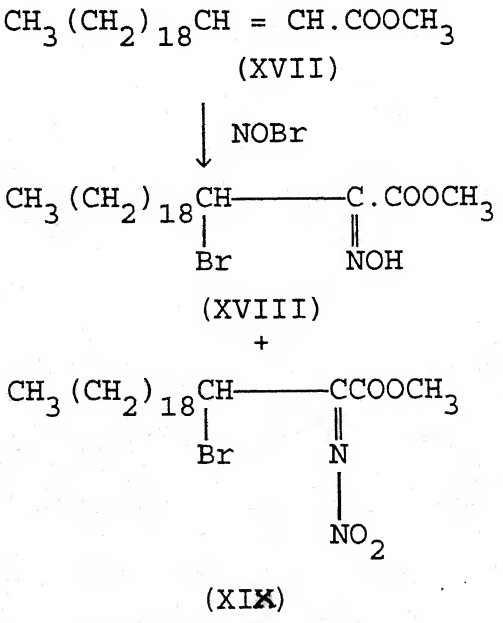


Further the results showed that nitrosobromination is the only primary reaction and the secondary products were formed as a result of two simultaneous pathways. Dimerization leads to product (XIII) and isomerization followed by oxidation yields an oxime (XIV) and nitrimine (IX). Dimerization seems to be much more feasible in methyl 10-undecenoate than in methyl oleate probably due to steric reasons. Isomerization of nitroso compound to an oxime also seems to be faster than in the case of methyl oleate as evidenced by the yields. Brominitrimine formation was found to be ~8% in yield when excess of nitrosyl bromide was used. None of the remainder is oxidized to the bromonitro compound apparently because of isomerization to bromooxime and subsequent oxidation to bromonitrimine.

Nitrosobromination of Methyl docos-*trans*-2-enoate (XVII)

Ethanol solution of methyl docos-*trans*-2-enoate was treated with NOBr (*in situ*) in a stoppered flask at 0-5°C by keeping in a refrigerator for about a month. Monitoring the reaction by TLC showed that the reaction is extremely slow and that sample from the reaction mixture after the work-up revealed the presence of three components, which were subjected to column chromatographic technique separation. The major component was found to be the starting material. Only about 10% of the compound (XVII) has reacted. The products were characterized on the basis of elemental analysis, IR and NMR.

Scheme 3



Characterization of the Compound (XVIII)

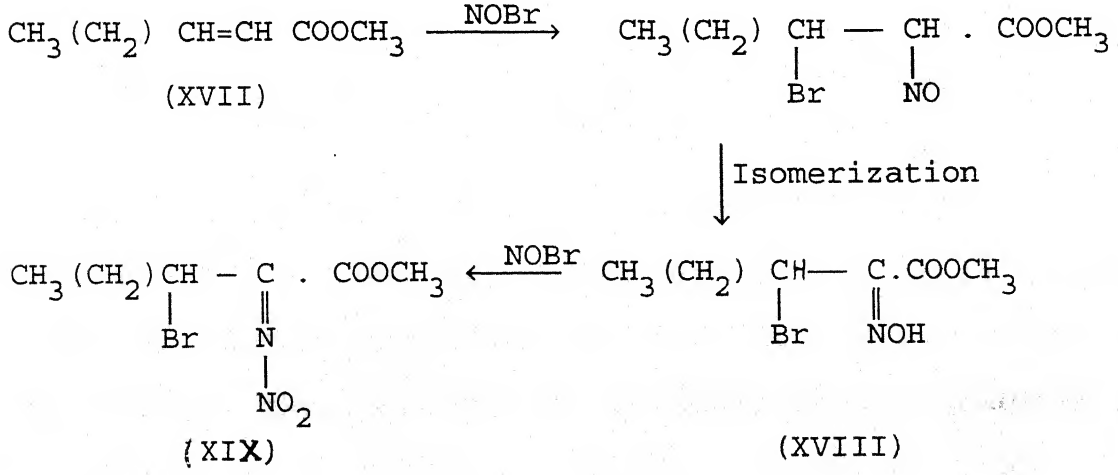
The compound (XVIII) gave satisfactory elemental analysis for $C_{22}H_{44}O_3NBr$ (positive Beilstein test). Compound (XVIII) gave informative IR spectrum with band at 3300 and 1640 cm^{-1} indicative of the oximino group. NMR spectroscopy was useful in confirming the structure of compound (XVIII) as methyl 2-oximino-3-bromodocosanoate. A signal was obtained for a single proton at τ 2.76 (signal disappeared on addition of D_2O) attributed to the oximino group proton (=N-O*H*). A triplet was observed at τ 6.16 for methine proton adjacent to bromine atom. The chemical shift and multiplicity of -C*H*Br- signal confirms the attachment of bromine atom to C-3 instead of C-2. Other proton signals were exhibited at τ 6.34 (s, 3H, $-\overset{\overset{O}{\|}}{C}-OCH_3$), 8.75 (br s, chain methylene protons) and 9.12 (distorted t, 3H, terminal group).

Characterization of the Compound (XIX)

The compound (XIX) was analyzed for $C_{23}H_{43}O_4N_2Br$. It responded to Beilstein test. Aside from elemental analysis, proof of structure for compound (XIX) was also obtained from spectroscopic evidences. The IR spectrum gave bands at 1640 (C=N), 1550 and 1360 (NO_2) cm^{-1} characteristic of nitrimino group. The NMR data were also consistent with the structure methyl 2-nitrimino-3-bromodocosonoate for the

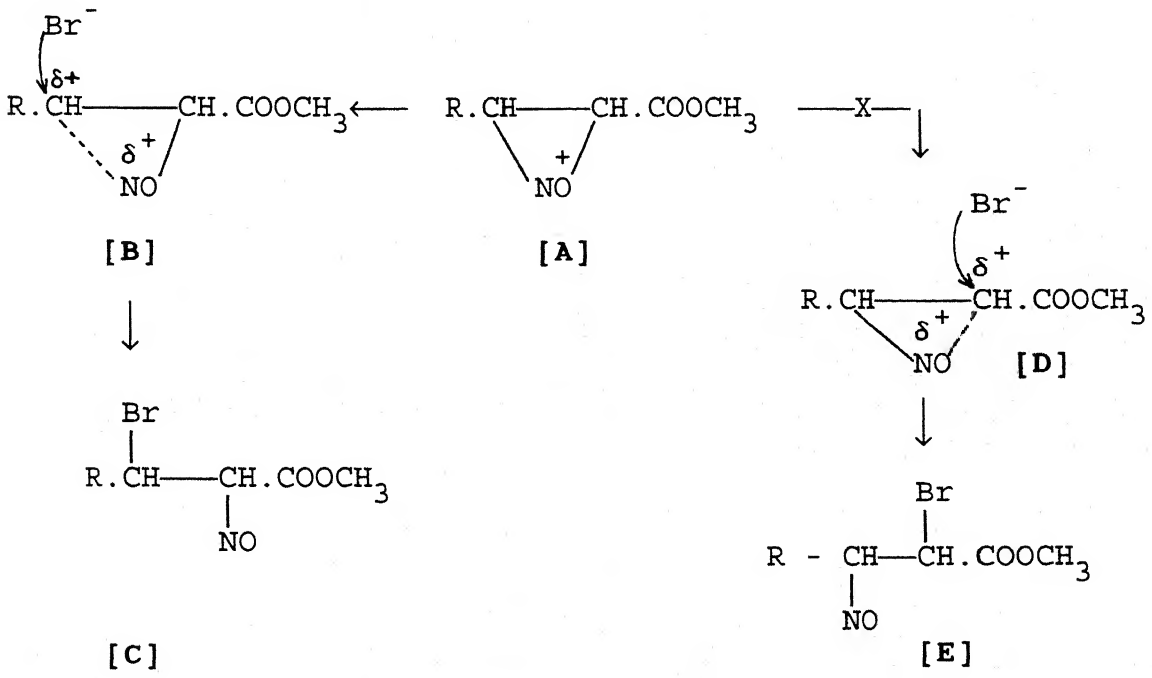
compound (XIX). It exhibited a triplet at τ 5.98 for methine proton adjacent to bromine atom (-C*H*Br-). Usually fatty ester signals were also observed at τ 6.36 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.65 (br s, chain methylene protons) and 9.1 (distorted t, 3H, terminal methyl). The chemical shift and multiplicity of methine proton signal adjacent to bromine atom confirms the attachment of bromine atom to carbon-3 as in the case of oximino compound (XVIII).

The formation of compound (XVIII) and (XIX) can well be explained through the nitrosobromination of compound (XVII) as the primary reaction. The isomerization of nitroso compound will give an oxime (XVIII) which on subsequent oxidation by NOBr will provide a nitrimine (XIX).



In case of α,β-unsaturated acid (XVII) only one isomer resulted during nitrosobromination. The presence of

electron withdrawing group ($-\overset{O}{\overset{\|}{C}}-OCH_3$) adjacent to double bond is involved in opening of nitrosonium ion intermediates. The electron withdrawing group will destabilize the transition state D relative to B and hence NO-carbonyl regiospecific NOBr adduct C will be formed.

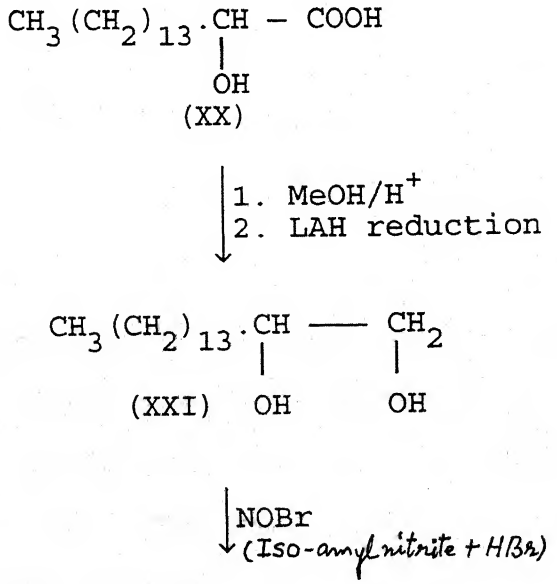


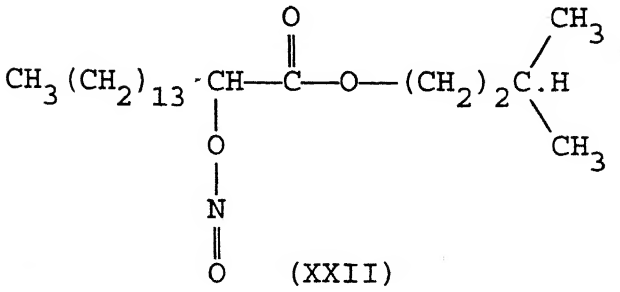
The considerable slow rate of the reaction is attributed to the proximity of the double bond to the electron withdrawing ester carbonyl function. Thus the decrease in the nucleophilic character of α,β-unsaturation slows down the electrophilic reaction of NOBr addition.

Reaction of Nitrosyl Bromide with Fatty 1,2-Diol (1,2-hexadecandiol, XXI)

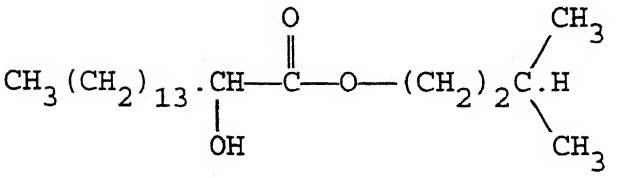
In the present work the diol used as a substrate was prepared from 2-hydroxyhexadecanoic acid (XX) which was obtained as one of the co-product during the preparation of C_{16} α,β-unsaturated acid. Alcoholic solution of 1,2-hexadecandiol (XXI) on treatment with an excess of nitrosyl bromide *in situ* at room temperature afforded a mixture of two products together with some unreacted compound as evidenced by analytical TLC. The components were resolved by silica column. Structures of compounds (XXII) and (XXIII) were corroborated by microanalysis, IR, NMR and Mass. The reaction carried out in the present investigaton is outlined in Scheme 4.

Scheme 4





(XXII)



(XXIII)

Characterization of Compound (XXII)

Microanalysis of the compound (XXII) supported the formula $C_{21}H_{41}NO_4$ (negative Beilstein test). IR spectrum exhibited band at 1630 cm^{-1} indicative of a nitrito group (C—O—N=O). IR band at 1730 cm^{-1} was also present showing the presence of an ester carbonyl group. NMR spectrum showed a two proton resonance at τ 5.9 (mc) corresponding to methylene group adjacent to oxygen atom (-O-C*H*$_2$-) and -C*H*O signal at τ 8.4 was exhibited for methine proton (-C*H*). A broad singlet at τ 8.4 was exhibited for methine proton (-C*H*). A broad singlet at τ 8.7 was observed for shielded methylene protons. NMR spectrum also showed an apparent doublet centered at τ 9.1 corresponding to three methyls. The structure of compound (XXII), as *iso*-amyl 2-nitritohexa-

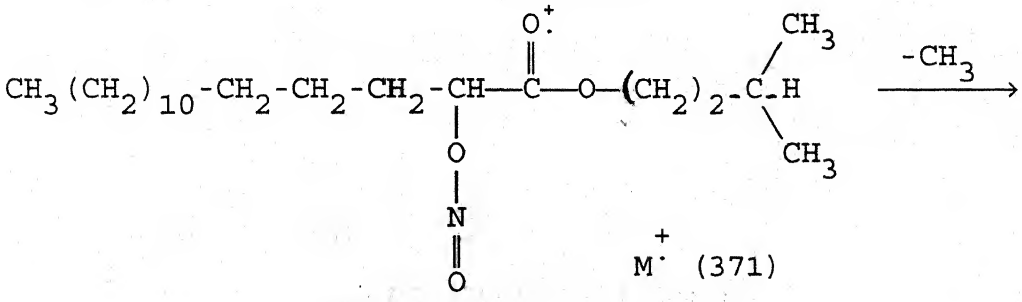
decanoate was further supported by mass spectrometry (Fig. 7, sheet VII). The genesis of important fragment ions are discussed.

The mass spectrum of compound (XXII) gave no molecular ion peak at m/e 371 ($C_{21}H_{41}NO_4$). The highest peak was observed at m/e 201 with other important peaks at m/e 191, 188, 187, 173, 157, 117, 87, 86, 85, 73, 72, 71 (base peak), 70, 69, 58, 57, 56 and 55 and other low mass ion species. The formation of some of the more significant ions can be rationalized according to schemes below. These fragmentation pathways are tentative since the mass spectra of appropriate deuterated analogs have been examined.

m/e 201 $\left[M^+.(371) - CH_3 - 155\right]$

This fragment ion which agrees with the loss of $-CH_3$ and loss of mass unit 155 from the expected molecular ion is shown in Scheme 5.

Scheme 5



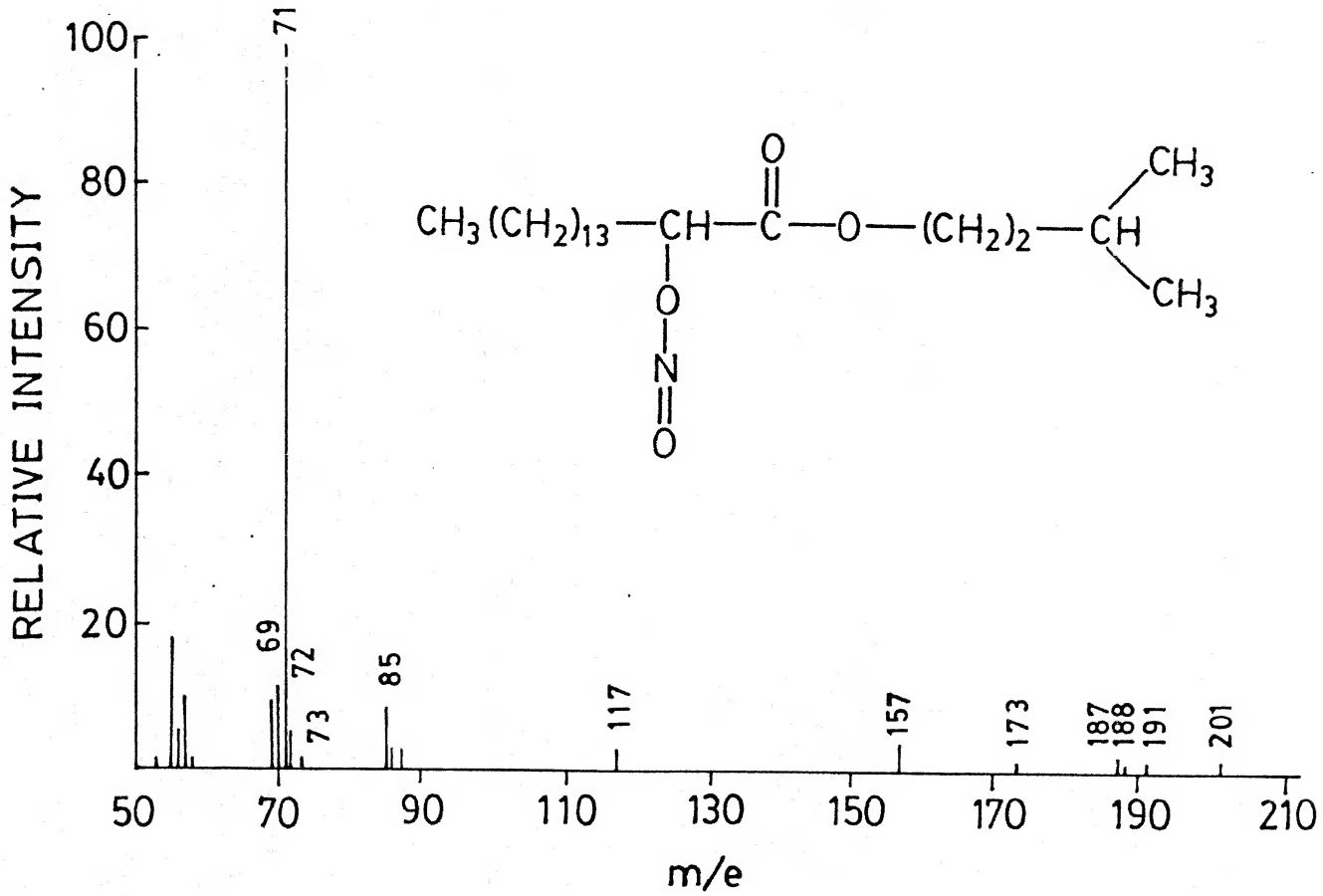
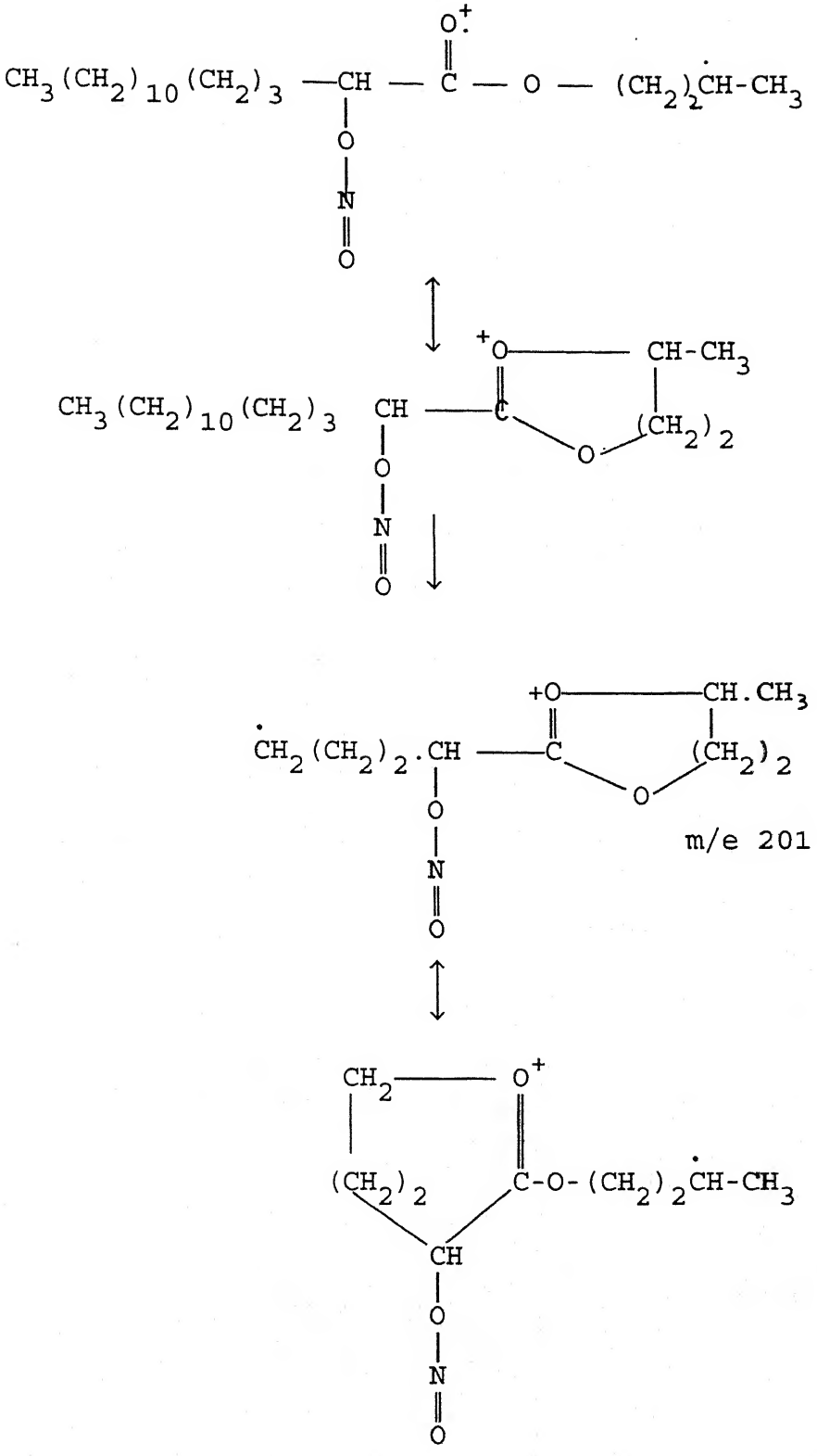


Fig.7. Mass spectrum of *iso*-amyl 2-nitritohexadecanoate (XXII)

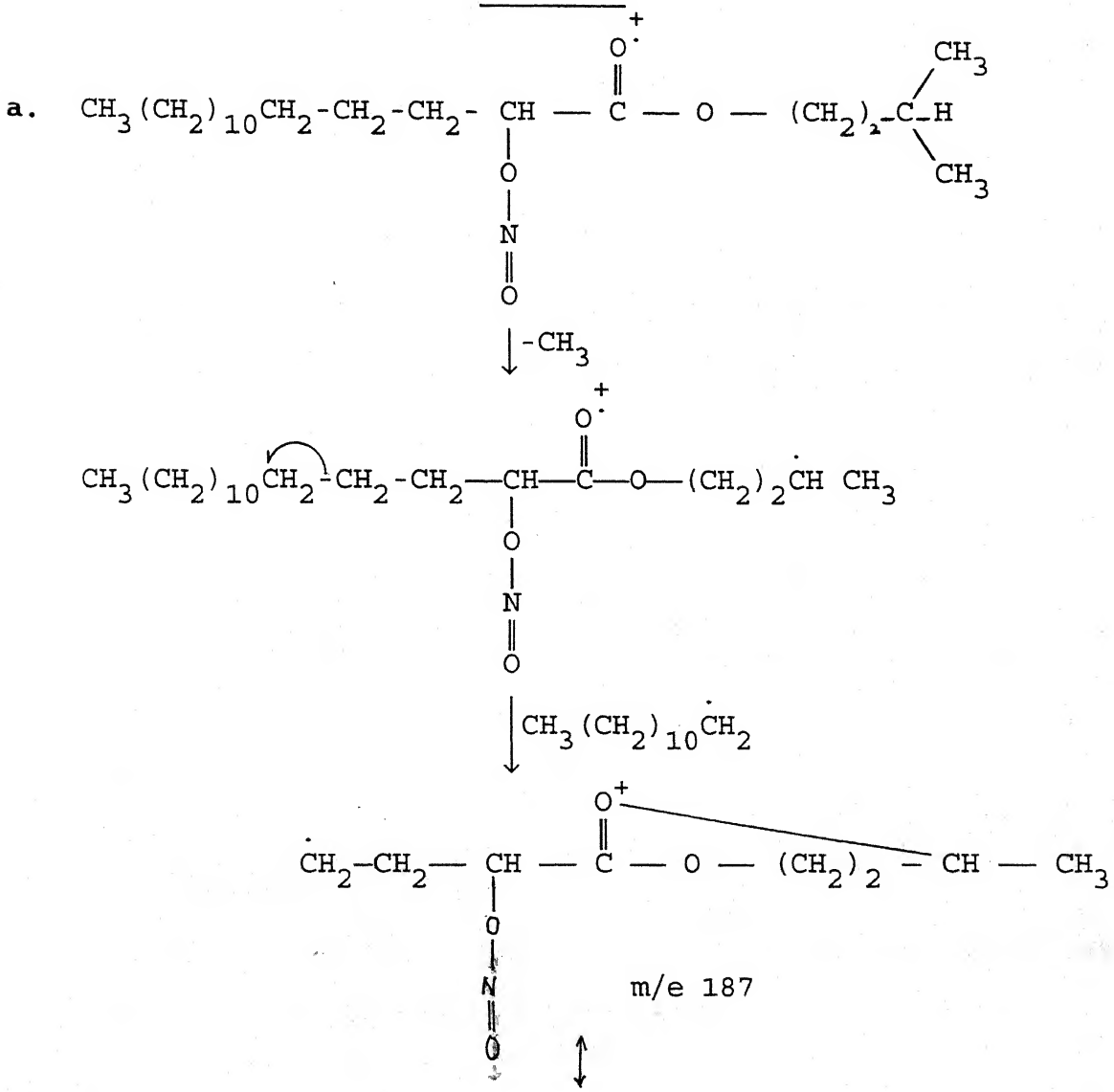


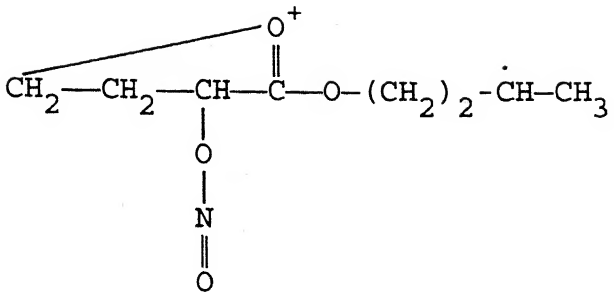
m/e 201

m/e 187 ($C_8H_{13}O_4N$) (M^+ - 184) or (m/e 201-14)

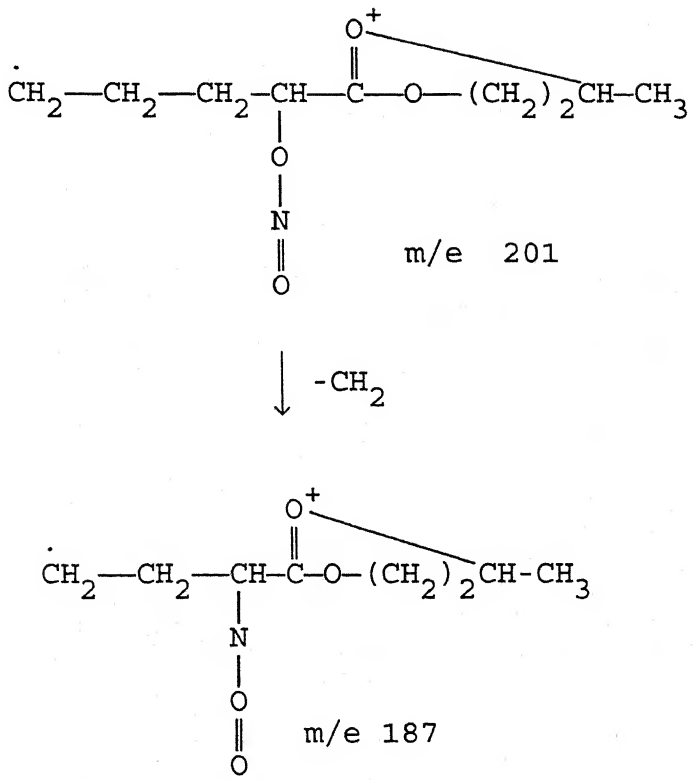
Obviously, this fragment ion is obtained either (a) by the loss of mass units 15 and 169 from the molecular ion or (b) by the loss of mass unit 14 from m/e 201 (Scheme 6).

Scheme 6





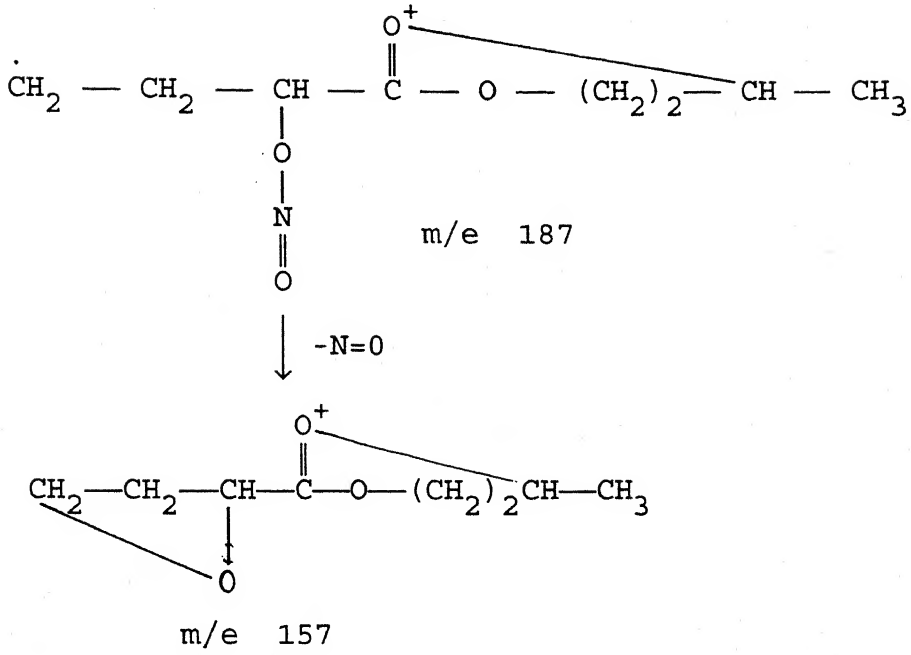
b. From Scheme 5 (201-14)



m/e 157 (m/e 187 - N = 0)

The fragment m/e 157 can be conveniently shown as arising from the ion m/e 187 (Scheme 7).

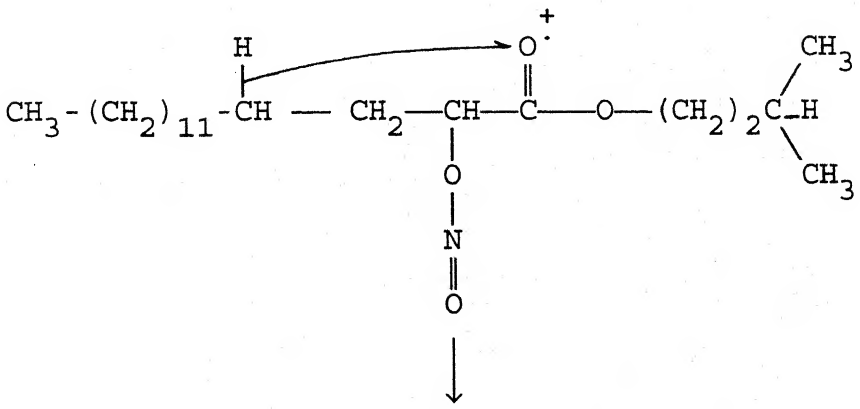
Scheme 7

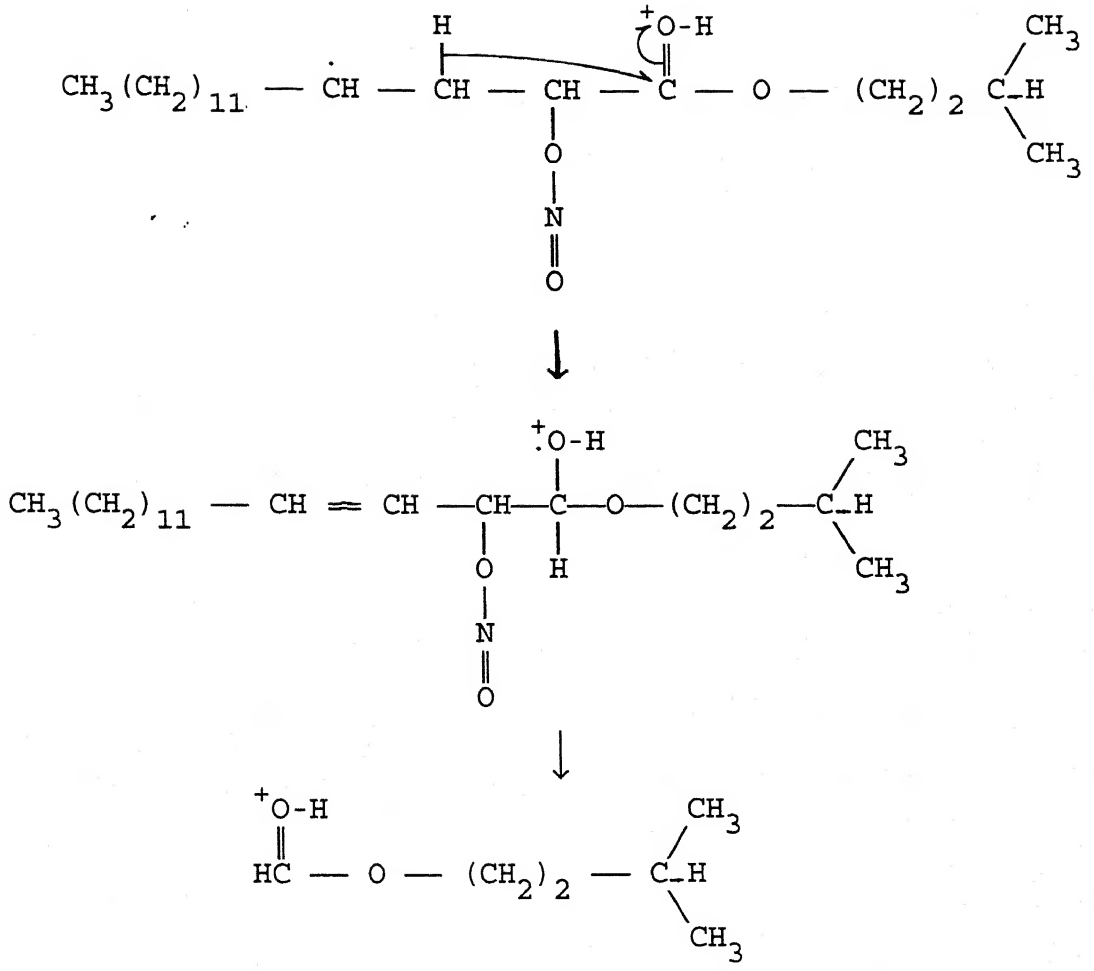


m/e 117

The formation of this ion may be rationalized according to scheme 8.

Scheme 8



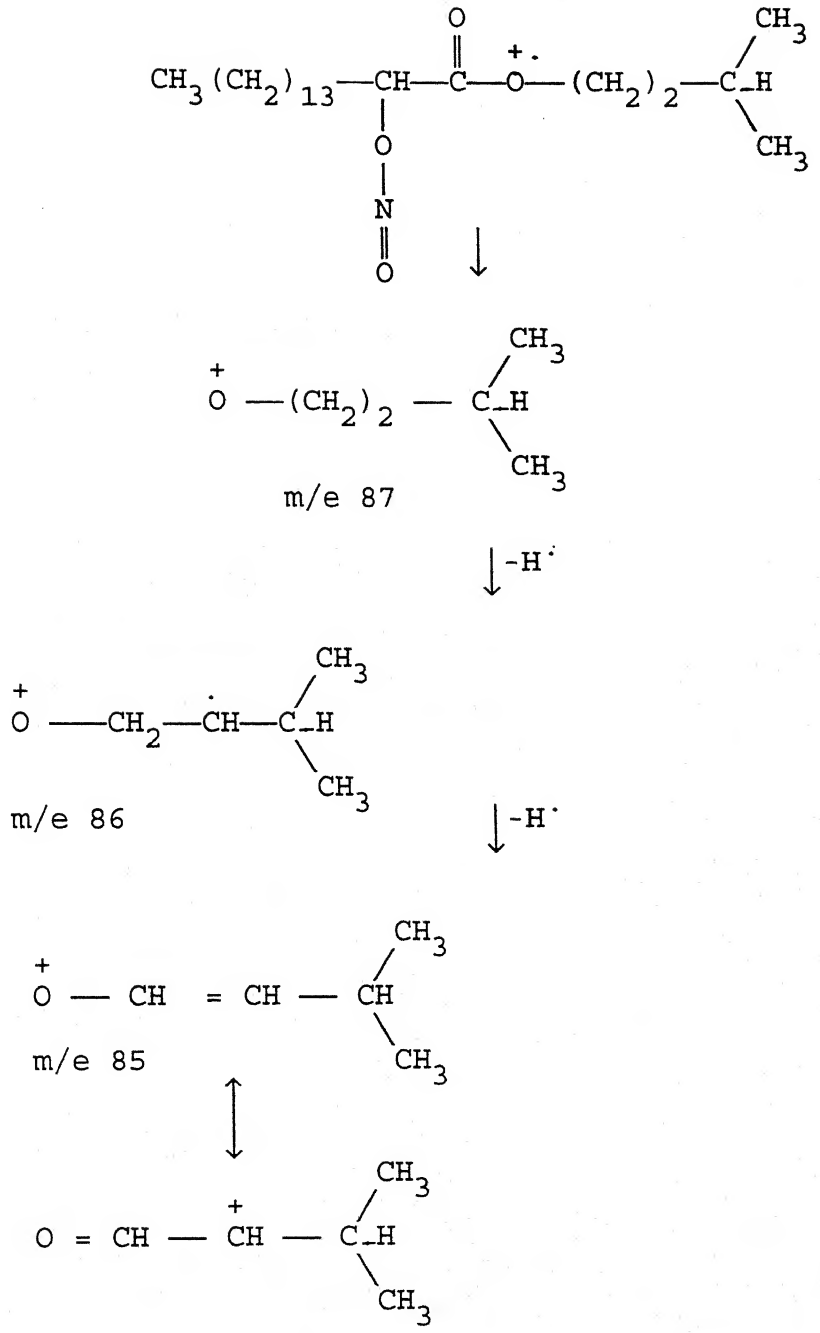


m/e 117

m/e 87, 86 and 85

The mass ion peaks at m/e 87, 86 and 85 may be explained to arise as below.

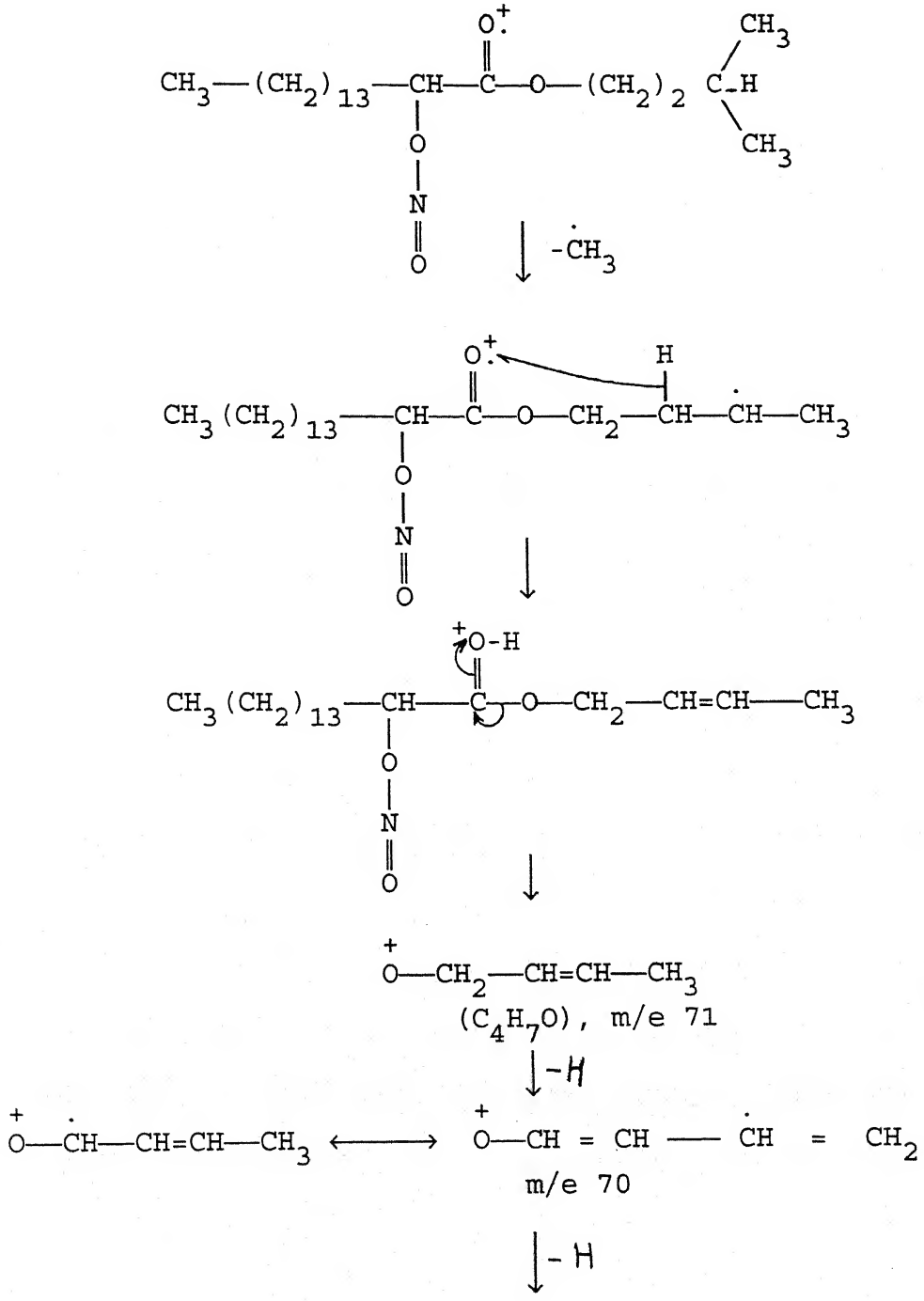
Scheme 9

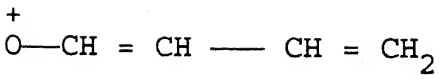


m/e 71 (base peak), 70 and 69

The fragment ion peak at m/e 71 constitute the base peak of the spectrum (Scheme 10).

Scheme 10



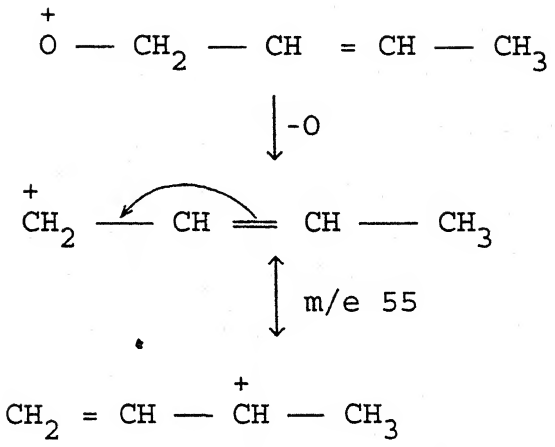


m/e 69

m/e 55

This hydrocarbon fragment ion may be shown to arise from the ion m/e 71 as below.

Scheme 11



Characterization of compound (XXIII)

The compound (XXIII) was analyzed correctly for $C_{21}H_{42}O_3$ (negative Beilstein test). IR spectrum displayed bands at 3350 cm^{-1} characteristic of hydroxyl group and at 1730 cm^{-1} due to ester carbonyl group. The NMR spectrum showed an apparent multiplet centered at τ 5.9 due to

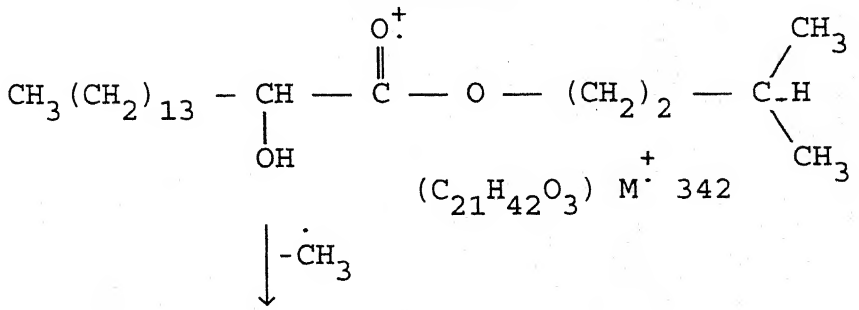
methylene protons adjacent to oxygen atom (-O-C*H*$_2$-) and -C*H*O, a signal at τ 8.4 due to methine proton (-C*H*$\langle^{CH_3}_{CH_3}$) and an apparent doublet centered at τ 9.05 due to methyl protons (9H). Abroad singlet at τ 8.65 for shielded methylenes was also observed. The NMR spectrum agrees well with the assigned structure for compound (XXIII) as *iso*-amyl 2-hydroxyhexadecanoate.

The mass spectrum of *iso*-amyl 2-hydroxyhexadecanoate (XXIII) (Fig. 8, sheet VIII) also gave no molecular peak at m/e 342 ($C_{21}H_{42}O_3$), but other significant peaks at m/e 327 (M-CH_3), 313, 295, 294, 286, 284, 225, 174, 133, 132 (base peak), 116, 113, 111, 109, 104, 100, 99, 97, 95, 90, 85, 84, 83, 82,81, 74, 71,70, 69, 68, 67, 57, 56, 55 and 54.

m/e 327 (M-CH_3)

The following mechanism has been proposed to account for the loss of CH_3 from the molecular ion m/e 342 (Scheme 12).

Scheme 12



Sheet-VIII

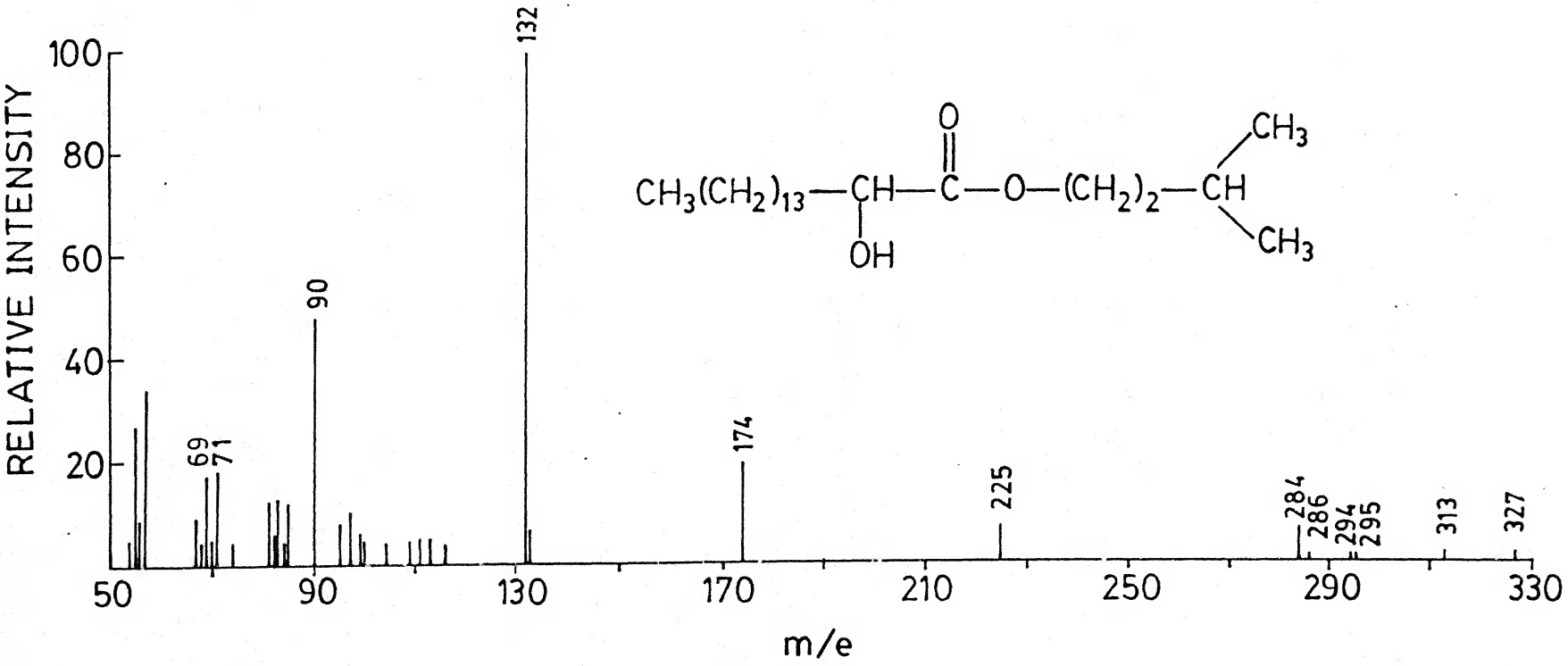
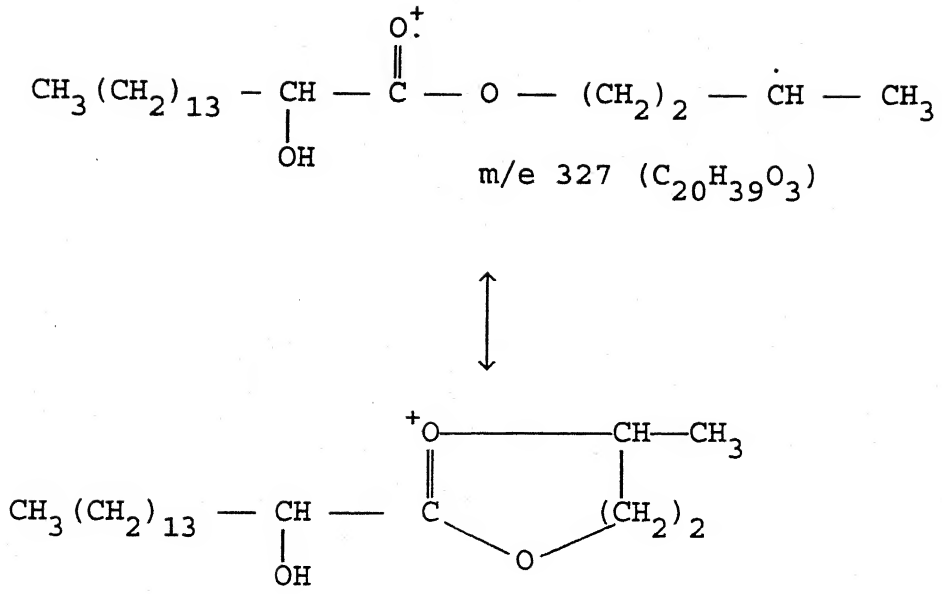


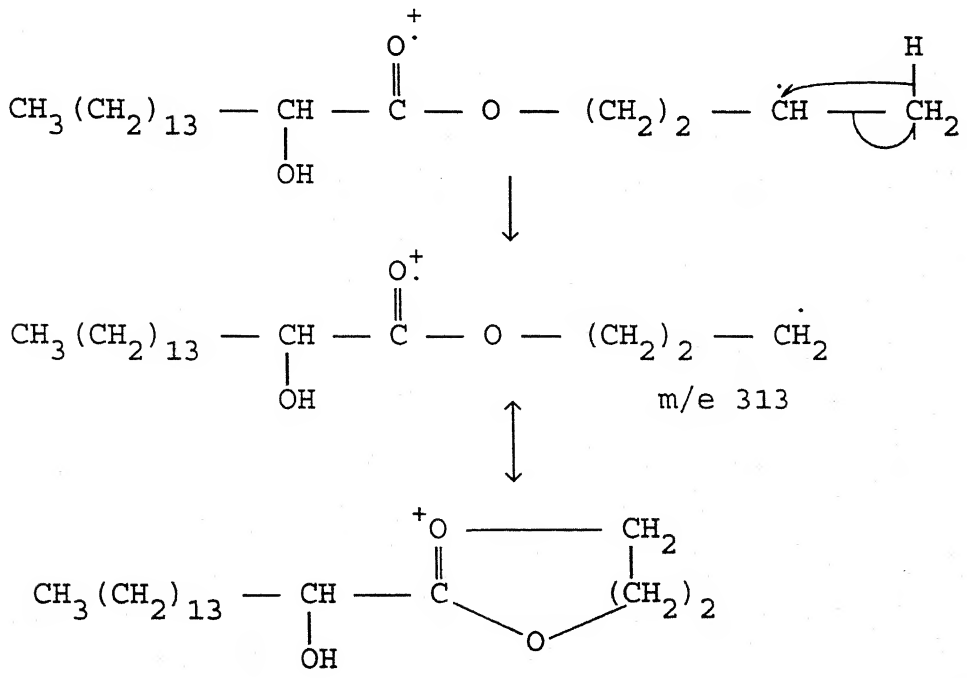
Fig.8. Mass spectrum of *iso*-amyl 2-hydroxyhexadecanoate (XXIII)



m/e 313 (m/e 327 - 14)

The fragment ion m/e 313 can be rationally derived from the ion m/e 327, as depicted in Scheme 13.

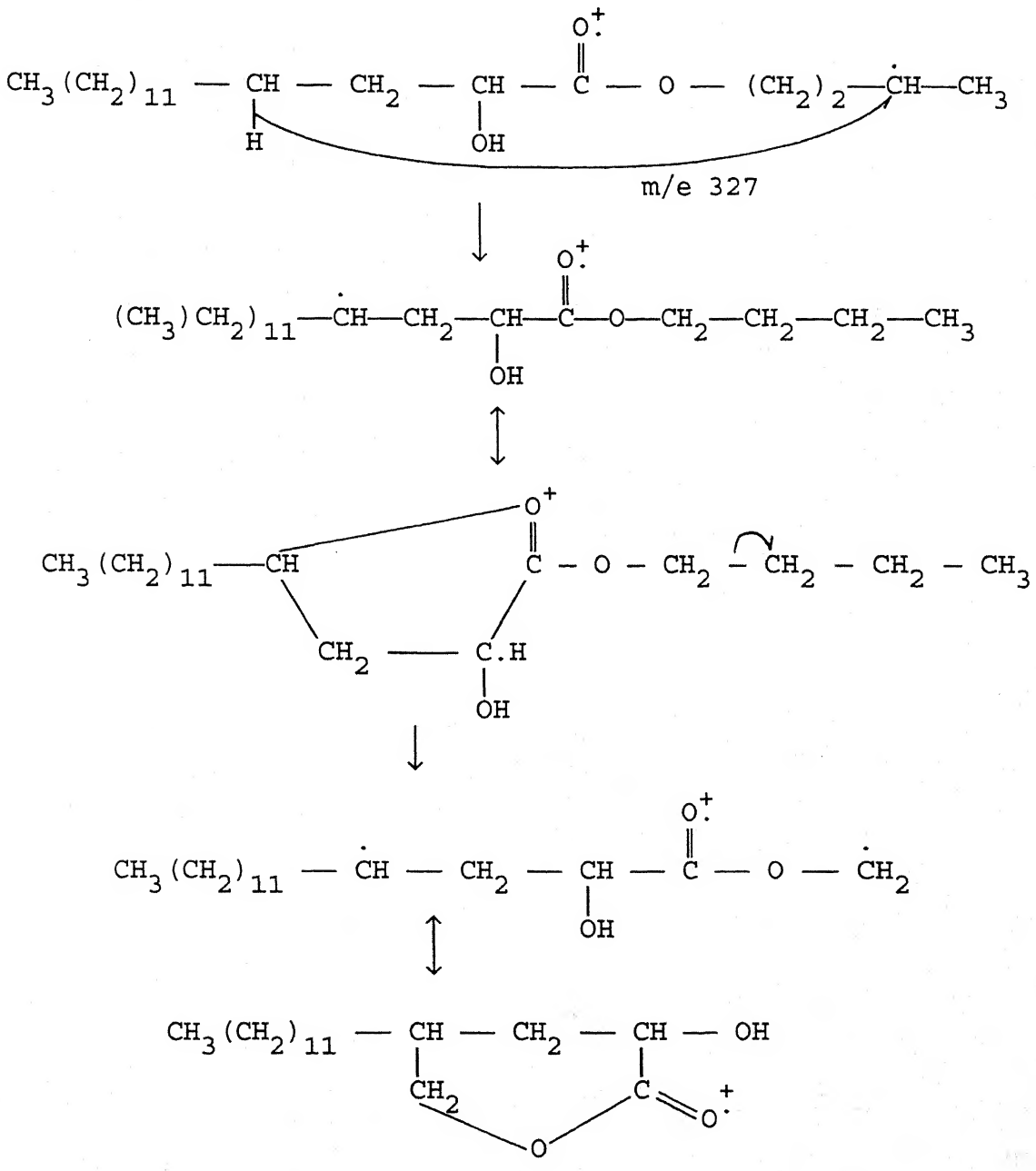
Scheme 13



m/e 284

The fragment ion m/e 284 may result by the loss of mass unit 43 from m/e 327 (Scheme 14).

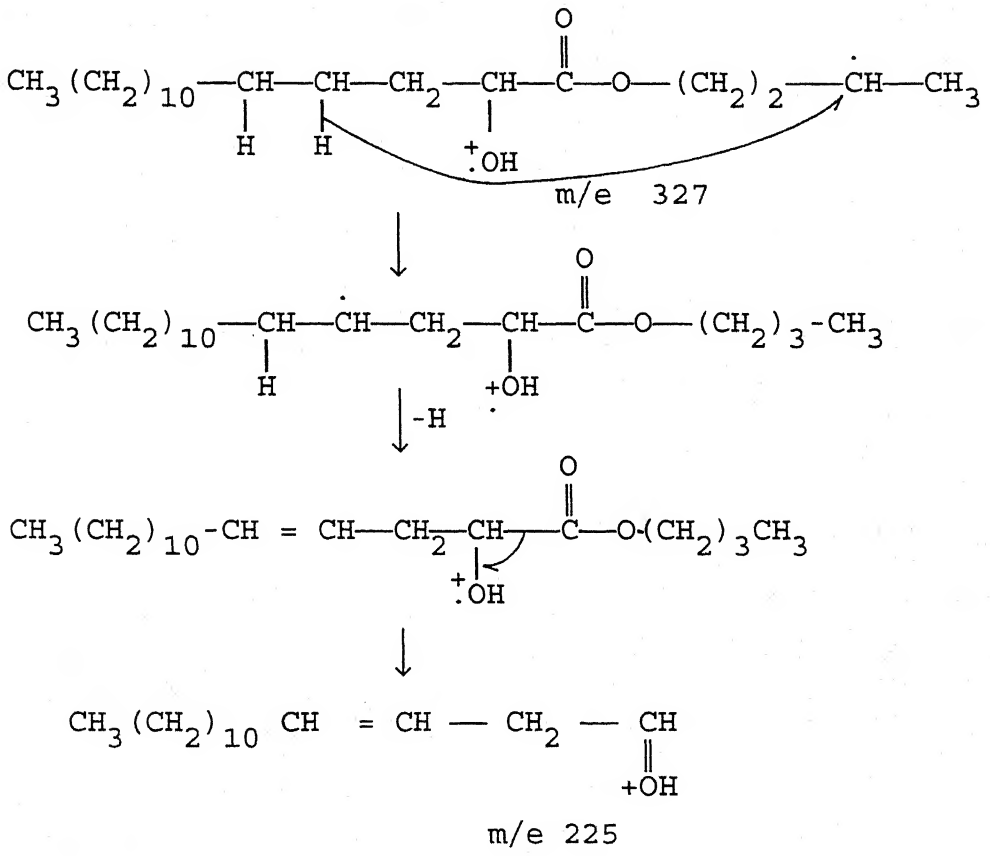
Scheme 14



m/e 225

The fragment ion m/e 225 is diagnostic in nature and it fixes the structure of compound (XXIII) as *iso*-amyl 2-hydroxyhexanoate. The ion formation may be attributed to the cleavage between carbon 1 and 3 (Scheme 15).

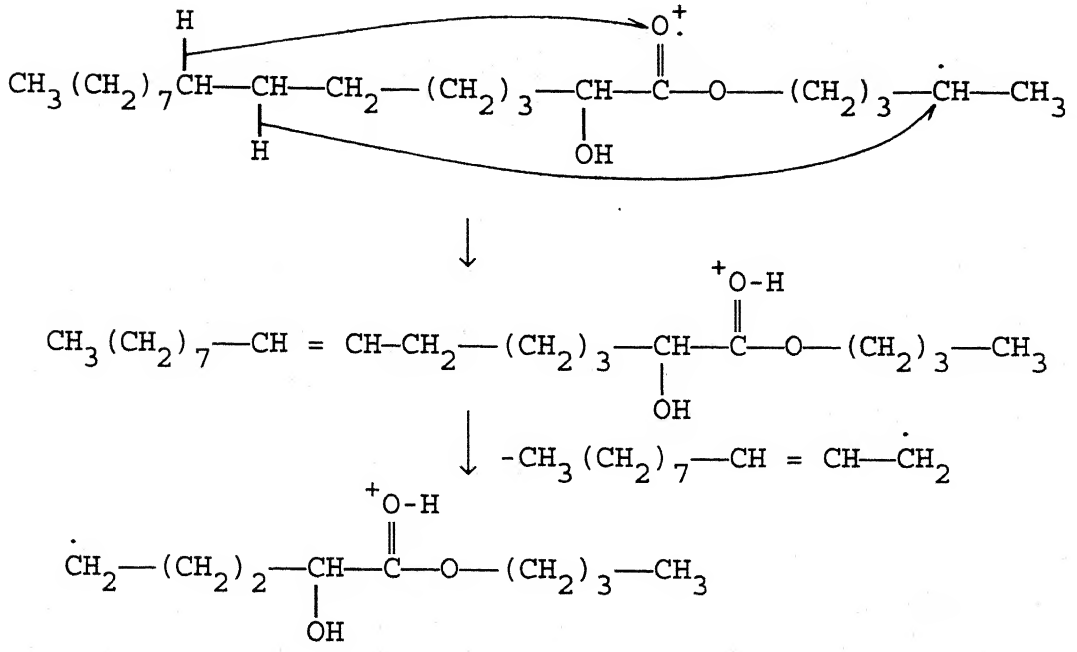
Scheme 15



m/e 174

The fragmentation peak at m/e 174 is fairly strong and this perhaps results by the loss of $CH_3(CH_2)_7CH{=}CH{-}\dot{C}H_2$ from the ion m/e 327 (Scheme 16).

Scheme 16



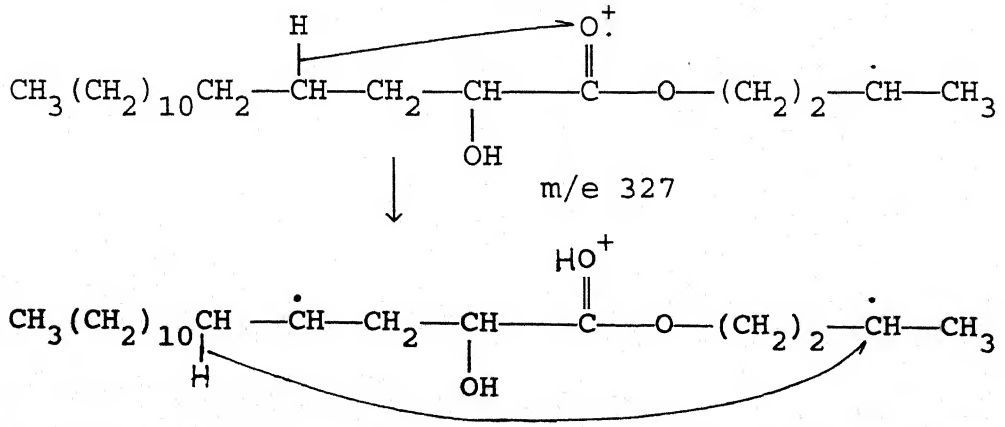
m/e 174

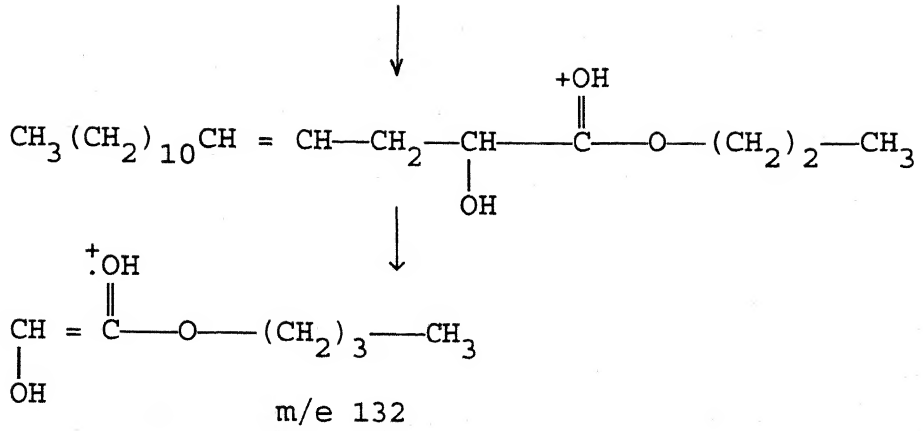
m/e 132

The fragment ion peak at m/e 132 constitutes base peak of the spectrum which indicates the position of the substituent -OH at C-2 atom of the chain. This fragment ion can be shown to arise by the migration of two hidrogens and simultaneous cleavage of 2,3 carbon-carbon single bond from m/e 327 (Scheme 17).

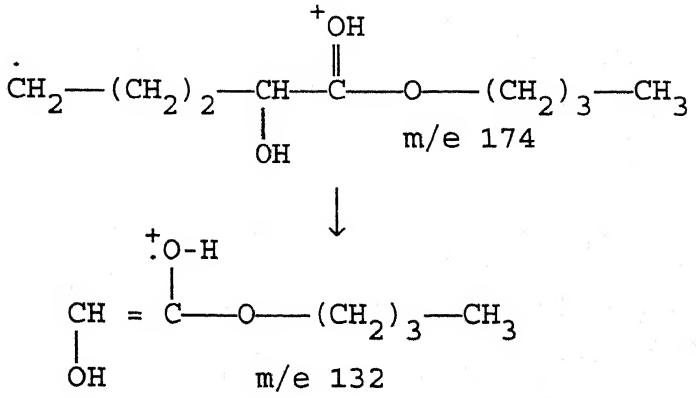
Scheme 17

a.





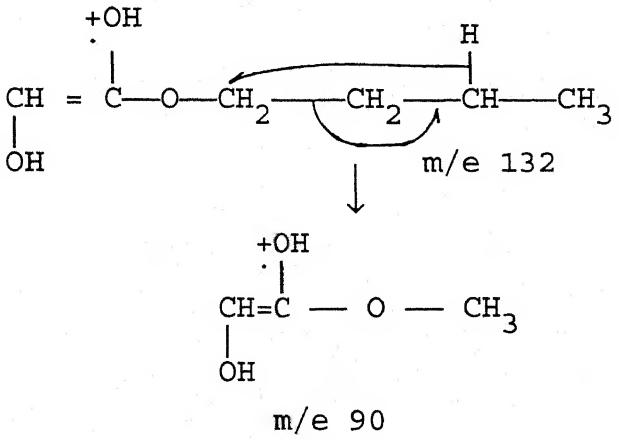
b.



m/e 90

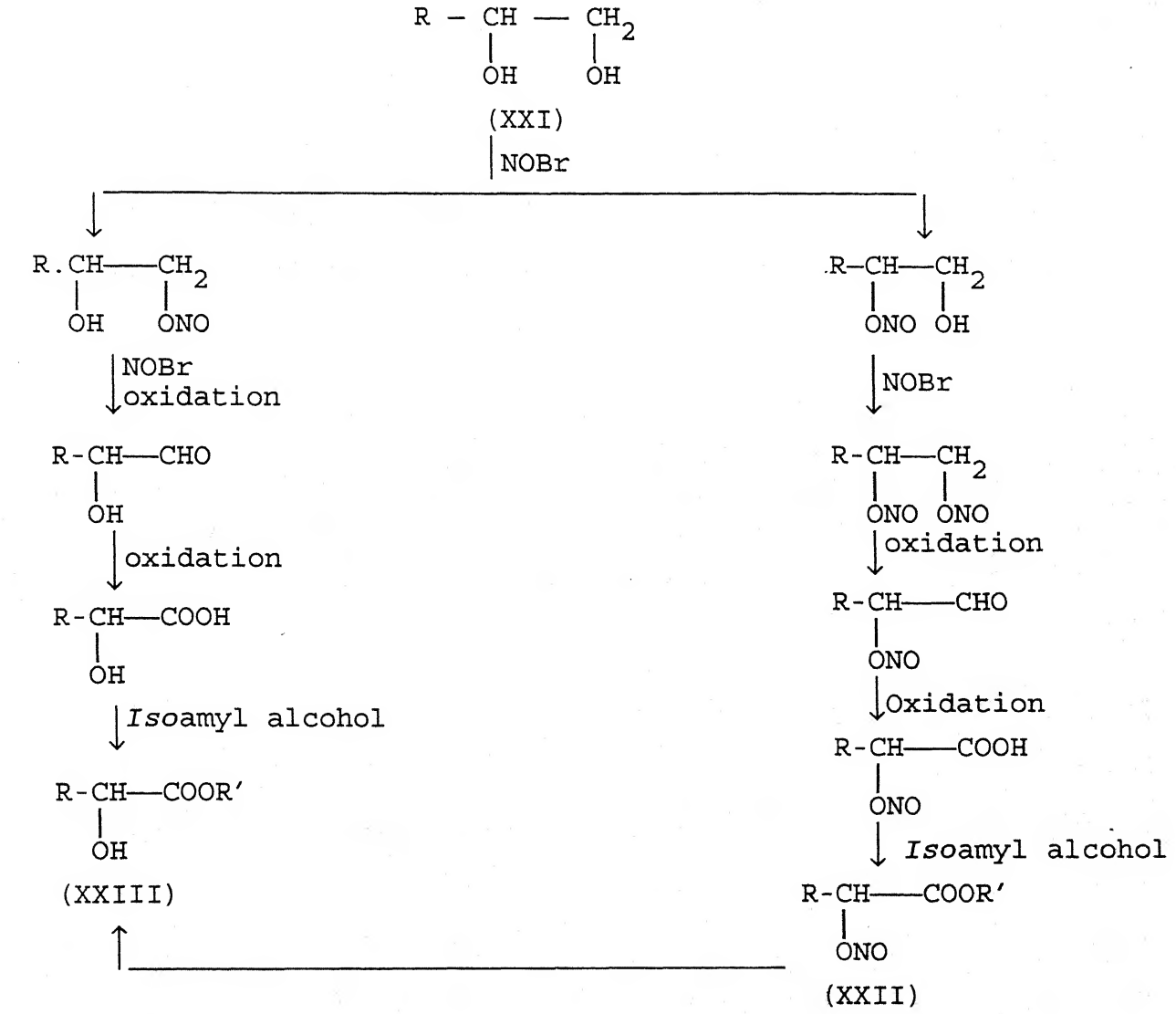
The fragment ion 90 may result by the loss of mass unit 42 from m/e 132 (Scheme 18).

Scheme 18



The probable route for the formation of compounds (XXII) and (XXIII) from (XXI) may be shown as under (Scheme 19).

Scheme 19



R = $CH_3(CH_2)_{13}$ —

R' = $(H_3C)(HC)CH—CH_2—CH_2—$

Reaction of nitrosyl bromide with 10,11-epoxyundecanoic acid (XXV)

It is known that the oxirane group is highly reactive and undergoes a wide variety of ring-opening reactions with a broad range of electrophiles and nucleophiles. During the last three decades, in particular, new and interesting reactions of the oxirane group have been described that provide new routes to other heterocyclic ring systems and functional groups.

The reaction of many reagents with oxirane group had been studied till date but no work had been reported on the reaction of oxiranes with nitrosyl bromide. A terminal epoxide was further selected for the present study with a view to study the direction of ring opening.

Preparation of 10,11-epoxyundecanoic acid (XXV) from 10-undecenoic acid (XXIV)

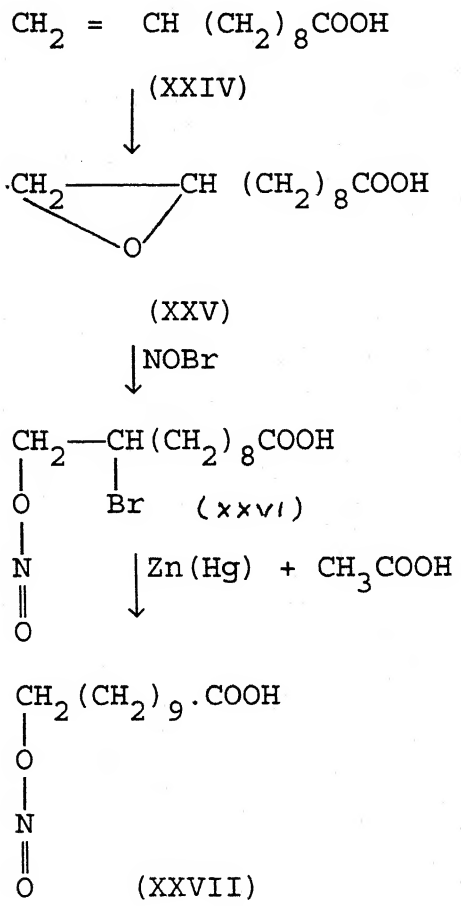
The 10-undecenoic acid (XXIV) was epoxidized to 10,11-epoxyundecanoic acid (XXV) by the procedure of Gunstone and Jacobsberg[110]. The epoxy acid (XXV) thus obtained was purified by using silica column.

Reaction of Compound (XXV) with NOBr

Nitrosyl bromide gas was slowly passed through alcoholic solution of 10-11-epoxy undecanoic acid at 0^{o} with continuous stirring till whole of the compound has reacted

as evidenced by TLC. Analytical TLC showed the quantitative yield of product (XXVI). A yellow oily liquid was obtained after the final work-up.

Scheme 20



The product (XXVI) responded Beilstein test. The elemental analysis corresponded to the molecular formula $C_{11}H_{20}O_4NBr$. The IR spectrum showed a strong band at 1630 cm^{-1} displayed by the nitrites. The NMR spectrum (Fig. 9, sheet (IX)) was decisive in arriving at a more firm conclusion regarding the structure of the compound as

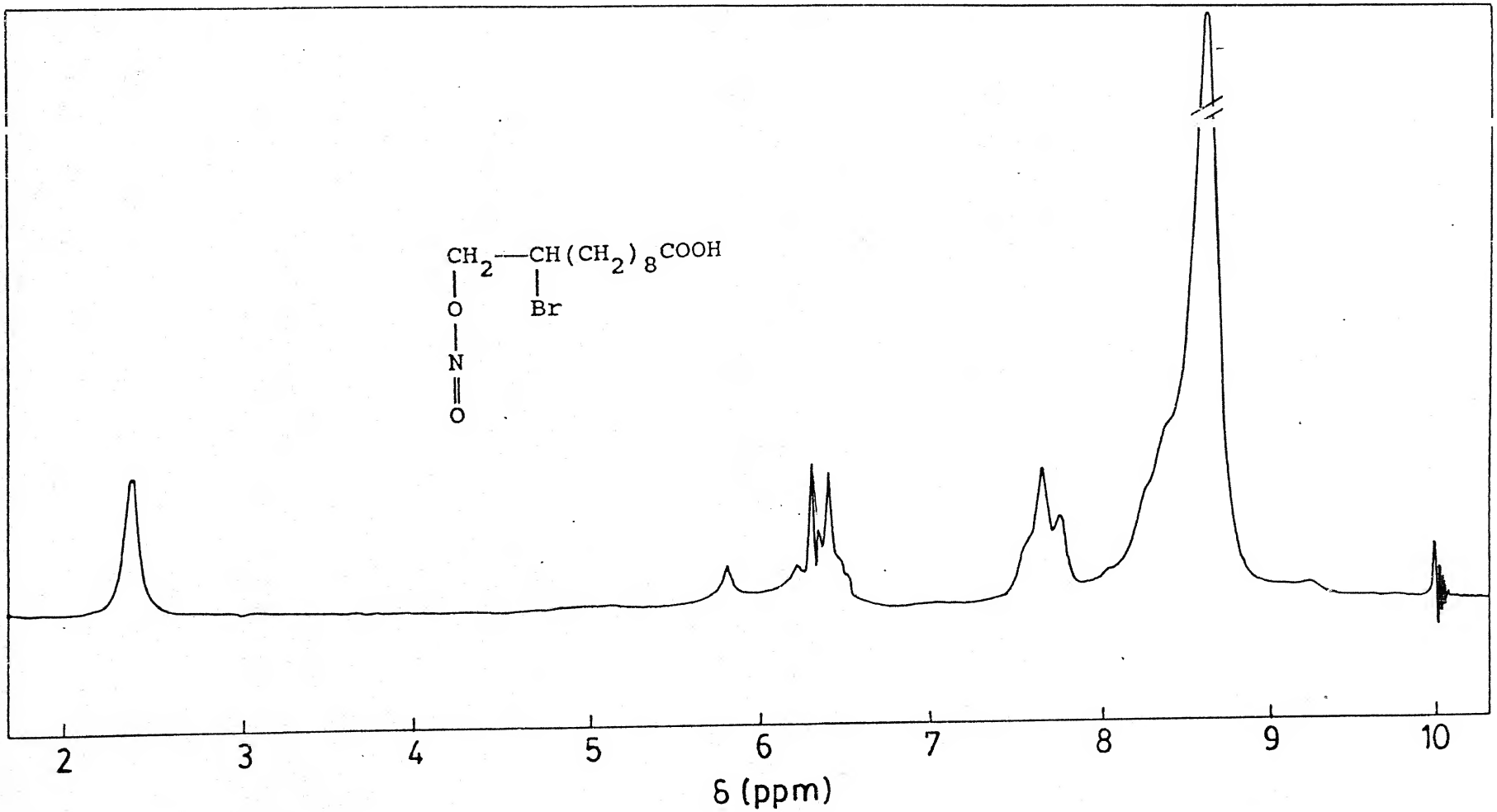
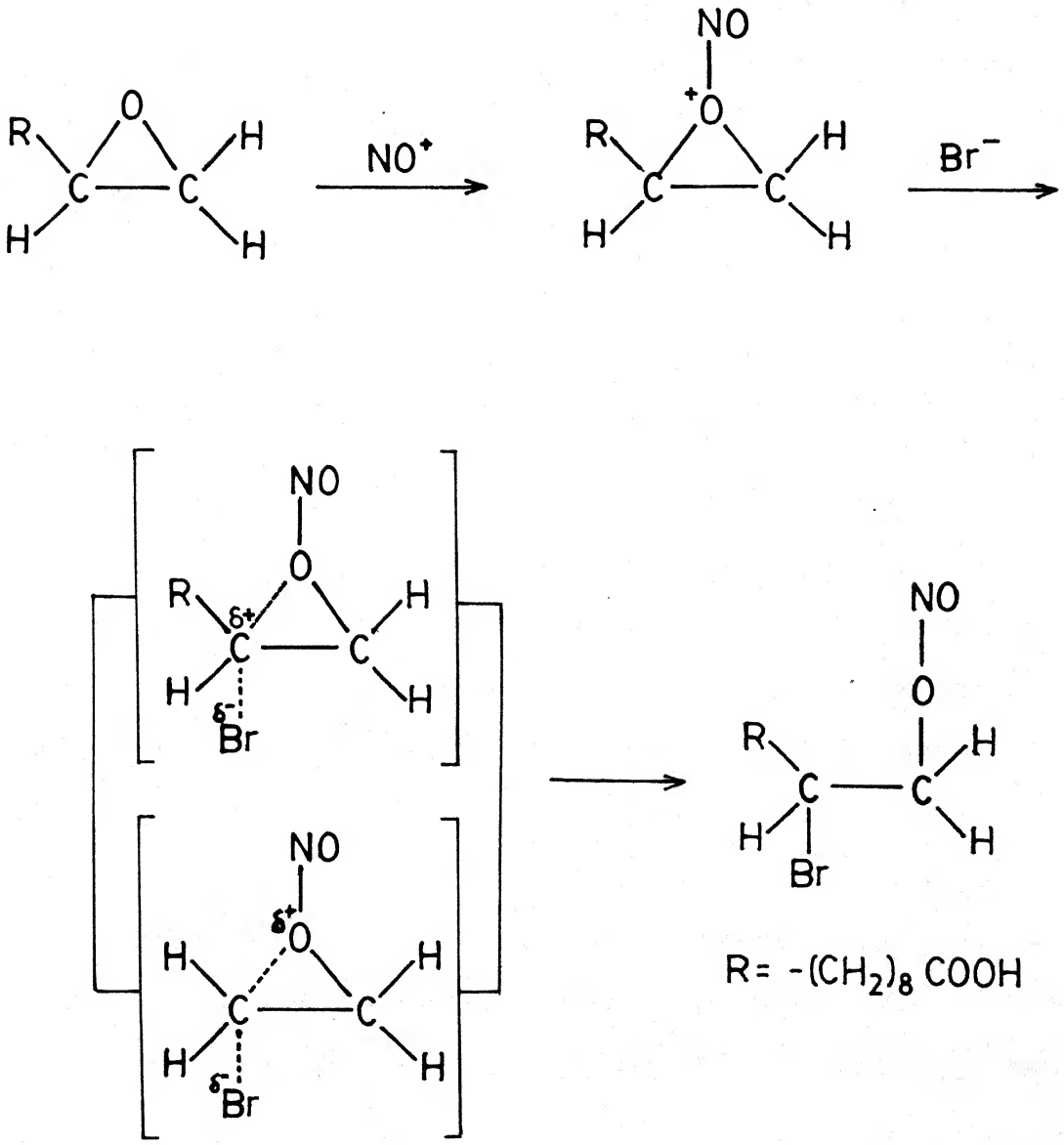


Fig.9. NMR spectrum of 10-bromo-11-nitritoundecanoic acid (XXVI)

10-bromo-11-nitritoundecanoic acid. NMR spectrum exhibited a doublet centred at τ 6.4 for two protons of methylene group adjacent to oxygen (–O–CH_2–) showing the attachment of nitrito group at terminal carbon atom. The methine proton of bromine-containing carbon displayed a signal at τ 6.12. Other usual NMR signals generally displayed by fatty acid chain were present at τ 2.47 (–C(=O)–OH, D_2O exchangeable), 7.7 (2H, protons α to carboxyl group), and 8.65 (br, s, shielded methylene protons). In order to ascertain the respective positions occupied by nitrito and bromo group the product (XXVI) was also subjected to reductive removal of bromine atom yielding a product (XXVII) showed the disappearance of signal at τ 6.12 showing thereby that the signal at τ 6.12 in compound (XXVI) was owing to the methine proton adjacent to bromine atom. These data supported the structure of compound (XXVI) as 10-bromo-11-nitritoundecanoic acid.

The exclusive formation of 10-bromo-11-nitrito isomer is in confirmity with the reported ring opening reactions of terminal epoxy compounds. A reasonable mechanism for the formation of compound (XXVI) from compound (XXV) is as follows.


R
O
H
C
C
H
H
NO+
NO
+O
Br−
δ+
δ−
Br
R= -(CH2)8 COOH

Experimental Procedure

Infrared (IR) spectra were obtained with a Perkin Elmer 1320 spectrophotometer. The abbreviations w, m and str stand for weak, medium and streching respctively. Ultraviolet (UV) spectra were determined with a Beckman DK-2A spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL PMX 60 NMR spectrometer. Chemical shifts are reported as τ (ppm) relative to tetramethyl silane (TMS). The samples were run as 10% solution in $CDCl_3/CCl_4$. The abbreviations s,d,t,q,m,um, mc, and br denote singlet, doublet, triplet, quartet, multiplet, unresolved multiplet, multiplet centred at and broad respectively. Mass spectra (MS) were measured with a Varian MAT-311 (A) mass spectrometer. All melting points were observed on a Kofler apparatus and are uncorrected. Microanalyses were performed by Chemistry Department, Indian Institute of Technology, Kanpur. Thin layer chromatographic (TLC) plates were coated with silica gel G, and a mixture of petroleum ether-ether-acetic acid (80:20:1, v/v/v) was used as developing solvent. The spots were visualized by charring after spraying with a 20% aqueous solution of perchloric acid or by exposing in iodine-chamber. Petroleum ether refers to a fraction of bp 40-60^{o}. Abbreviations Anal. and Calcd. stand for analysis and calculated respectively.

Preparation of nitrosyl bromide *in situ*

The nitrosyl bromide was generated *in situ* by the action of hydrobromic acid on *iso*-amyl nitrite[2]. *Iso*-amyl nitrite was prepared as under: In a 3l three-necked round-bottomed flask, fitted with a mechanical stirrer, a separating funnel extending to the bottom of the flask, and a thermometer, were placed 380g (5.5 moles) of C.P. $NaNO_2$ and 1.5l of water. The flask was surrounded by an ice-salt mixture, and the solution was stirred until the temperature falls to 0^{o}. A mixture of 100 ml of water, 136 ml (250 g, 2.5 moles) of conc. H_2SO_4 (sp. gr. 1.84), and 440 g (5 moles) of commercial *iso*-amyl alcohol was cooled to 0^{o} and by means of the separating funnel was introduced slowly beneath the surface of the nitrite solution, with stirring. The alcohol solution was added slowly enough so that practically no gases evolved, and temperature was kept at $\pm 1^{o}$ from $1\frac{1}{2}$ to 2 hr.

The resulting mixture was allowed to stand in the ice-salt bath until it separated into two layers, and the liquid were decanted from the sodium sulphate into a separating funnel. The lower aqueous layer was removed and the *iso*-amyl nitrite layer washed twice with 50 ml portions of solution containing 2 g $NaHCO_3$ and 25 gm of NaCl in 100 ml of water. After drying over 20 g of anhydrous sodium sulphate, the yield of practically pure *iso*-amyl-nitrite

amounted to 81-85% of the theoretical amount.

Nitrosobromination of methyl oleate

Preparation of methyl oleate (VII)

Pure oleic acid (10.0 g) was disolved in anhydrous methanol (100 ml) containing catalytic amount of sulphuric acid and refluxed for $1\frac{1}{2}$ hr. The mixture was then diluted with water and extracted with ether. The ethereal extract was dried over anhydrous sodium sulphate. Evaporation of ether yielded methyl oleate (VII) as a colourless oil (9.8 g).

Reaction of methyl oleate with approximately stoichiometric quantity of NOBr

A mixture of 3g (0.01 mole) of methyl oleate and 1.5 g (0.012 mole) of *iso*-amyl-nitrite in 50 ml of alcohol was cooled to about 0^{o} in an ice-salt bath. 1.6 ml of conc. HBr was added dropwise with stirring in 30 minutes. Strring was continued at ice bath temperature for 1.5 hr. A green colour solution was obtained. The reaction mixture was washed with water dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The generated *iso*-amyl alcohol was removed by partitioning between 70% methanol and petrol (1:1). The petrol fraction was dried over anhydrous sodium sulphate and the products

obtained after evaporation of the solvent were chromatographed over a column of silica gel (50 g). Elution with petroleum ether-ether (98:2, V/V) gave Fraction 1. This fraction was obtained in major amount as a green liquid. The spectral and combustion data of this fraction are tabulated below:

Anal. Calcd. for $C_{19}H_{36}NO_3Br$: C, 56.1; H, 8.87; N, 3.44
Found: C, 56.9, H, 8.96; N, 3.46

IR (neat): 3450 (w, OH), 1730 (ester-$\overset{O}{\overset{\|}{C}}$-), 1640 (w, C=N), 1570 (N=0), 1110 (C-N), 540 (C-Br) cm^{-1}.

NMR (CCl_4): τ 2.52 (= NO*H*, D_2O exchangeable), 6.14 (1H, -C*H*Br-), 6.34 (s, 3H, $\overset{O}{\overset{\|}{C}}$-OC$H_3$), 6.65 [1H, -C*H*(NO)-], 7.76 (2H, methylene protons α to ester group), 8.65 (2H, methylene protons), and 9.12 (distorted t, 3H terminal methyl protons).

Subsequent elution with petroeumether-ether (95:5, V/V) gave fraction 2. This fraction was obtained in minor amount (R_f 0.3). The combustion and spectral data are given below:

Anal. Calculated for $C_{19}H_{36}NO_3Br$: C, 56.1; H, 8.87; N, 3.44.
Found: C, 56.5, H = 8.89, N = 3.47

IR (neat) 3450 (OH), 1730 (ester-$\overset{O}{\overset{\|}{C}}$-), 1640 (m, C=N), 540

(C-Br) cm^{-1}.

NMR ($CDCl_3$): τ 2.65 (1H, = NO*H*), 6.18 (1H, -CHBr-), 6.35 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 7.76 (2H, methylene protons α to the ester group), 8.65 (br s, shielded chain methylene protons) and 9.12 (distorted t, terminal methyl protons).

Treatment of methyl oleate with excess of NOBr (*in situ*)

The methyl oleate (VII) on treatment with excess of NOBr (*iso*-amyl-nitrite + HBr) produced a compound (X) in addition to compound (VIII) and (IX). The reaction product was worked up as described earlier. The compound (X), having R_f value (0.5) higher than oxime (IX), was characterized as methyl 9(10)-bromo 10(9)-nitriminostearate. The spectral and combustion data of compound (X) are given below:

Anal. Calcd. for $C_{19}H_{35}N_2O_4Br$: C, 52.41; H, 8.04; N, 6.43. Found: C, 52.43; H, 7.98; N, 6.45.

IR (neat): 1730 (s, ester $-\overset{O}{\overset{\|}{C}}-$), 1640 (m C=N), 1550 and 1360 (NO_2), 550 (C-Br) cm^{-1}.

NMR (CCl_4): τ 6.12 (1H, -C*H*Br), 6.34 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 7.38 [t, 2H, $-CH_2-C(=N.NO_2)-$], 7.76 (t, 2H, $-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.67 (br s, shielded methylenes), 9.12 (distorted t, 3H, terminal methyl).

Nitrosobromination of methyl 10-undecenoate (XI)

Preparation of methyl 10-undecenoate (XI)

Methyl 10-undecenoate (XI) was prepared by refluxing the acid (10.0 g) with absolute methanol (100 ml) and catalytic amount of sulphuric acid as described previously.

Nitrosobromination

To the solution of methyl 10-undecenoate (XI, 2.5 g) in 100 ml ethanol was added 2.0 g of *iso*-amyl nitrite and cooled at about 0^{o} in an ice-salt bath. 2.5 ml of conc. HBr was added dropwise with stirring in 30 min. Stirring was continued at ice-salt temperature for 2 hr. After the usual work-up the products showed the presence of four components on TLC and were chromatographed over a column of silica gel (40 g). Only three components were isolated and characterized in pure form.

Elution with petroleum ether gave a blue coloured liquid which was not analyzed further on account of being unstable.

Subsequent elution with petroleum ether-ether (90:5, V/V) gave methyl 10-bromo-11-nitriminodecanoate (XV, 0.4 g) as a green oil.

Anal. Calcd. for $C_{12}H_{21}N_2O_4Br$: C, 42.72; H, 6.23; N, 8.30. Found: C, 42.75; H, 6.28; N, 8.32

IR (neat): 1730 (ester -C-), 1630 (C=N), 1550 (NO) and 530 (C-Br) cm^{-1}.

NMR (CCl_4): τ 3.84 (1H, -C*H* = N.NO_2), 5.46 (m, 1H, -C*H*Br-), 6.34 (s, 3H, -C–OCH_3), 7.76 (2H, -C*H*$_2$-C-OCH_3), 8.68 (br s, shielded methylene). Subsequent elution with a mixture of petroleun ether-ether (90:10, V/V) gave methyl 10-bromo-11-oximinoundecanoate (XIV; 0.5 g mp 57°).

Anal. Calcd. for $C_{12}H_{22}O_3NBr$:C, 46.75; H, 7.14; N, 4.54. Found:C, 46.75; H, 7.16; N, 4.57.

IR (In Nujol): 3300 (OH), 1730 (ester-C-), 1680 (C=N), 540 (C-Br) cm^{-1}.

NMR ($CDCl_3$): τ 2.6 (1H, =N-OH), 3.6 (1H, -CH = N-OH), 5.72 (1H, -C*H*Br-), 6.34 (s, 3H, -C-OC*H*$_3$), 7.76 (2H, CH_2 α to the ester -C- group, 8.65 (brs, chain-C*H*$_2$-).

Further support to the structure of compound (XIV) was achieved from the analysis of the corresponding carbonyl compound (XVI) obtained by the deoximation of the compound (XIV).

Deoximation of compound (XIV) by levulinic acid and HCl[109]

200 mg of the oxime was mixed with 30 parts of a solution of 9 volumes of levulinic acid and one volume of 1N

HCl. This mixture was placed in a earlenmeyer flask and stirred at room temperature for 3 hr. If the oxime was not immediately soluble, it gradually dissolved over the course of the 3 hr. The solution was then diluted with water, extracted with methylene chloride, and the extracts washed free of levulinic acid with bicarbonate solution. The methylene chloride was removed and the carbonyl compound recovered by chromatography.

IR (neat): 2910, 2840, 2800, 1730, 1710 (ester and aldehyde carbonyls), 1450, 1370, 540 (C-Br) cm^{-1}.

Subsequent elution with a mixture of petroleum ether-ether (60:40, V/V) gave a dimer of methyl 10-bromo-11-nitrosoundecanoate (XIII, 0.8 g, m.p. 98°).

Anal. Calcd. for $(C_{12}H_{22}O_3NBr)_2$: C, 46.75; H, 7.14; N, 4.54. Found: C, 46.77; H, 7.18; N, 4.56.

IR (In Nujol): 2910, 2840, 1725 (ester $-\overset{O}{\overset{\|}{C}}-$), 1450, 1370, 1270, 1205, 1180, 1160, 540.

NMR ($CDCl_3$): τ 5.46 (6H, -*CH*Br- and $-CH_2-\overset{O}{\overset{|}{N}}-$), 6.34 (s, 6H, (s, 6H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 7.77 (protons α to the ester $-\overset{O}{\overset{\|}{C}}-$), 8.67 (br, s, chain methylene).

Preparation of α, β-unsaturated Fatty Acid

The docos-*trans*-2 enoic acid was prepared from

docosanoic acid by the method of Palameta and Prostenik[111].

General Procedure

To a well stirred mixture of the saturated acid (25 g) and red phosphorous (1.15 g), dry bromine (12.5 ml) was added dropwise at 90° in a period of 7 hr. The mixture was vigorously stirred during the addition of bromine by using a mercury sealed stirrer. Heating was continued for 24 hr and the cooled solution was poured into cold water and left overnight. The solid product was filtered, extracted with ether, washed with 10% aqueous sodium sulphite solution, then with distilled water and dried over anhydrous sodium sulphate. The 2-bromo-acid obtained after evaporation of the ether was heated under reflux with powdered potassium iodide (24 g) in 95% ethanol (175 ml) for 6 hr. To the cooled solution potassium hydroxide (16 g) was added and the mixture was refluxed for another 4 hr. Most of the alcohol was removed under reduced pressure and the residue diluted with water, acidified with dilute hydrochloric acid, and extracted with ether. The combined ether extracts were washed with water and dried over anhydrous sodium sulphate. After evaporation of the solvent, a mixture of α,β-unsaturated and their co-products, i.e., 2-hydroxy and 2-ethoxy acids were obtained.

The 2-hydroxy acid was separated from α,β-unsaturated acid as a copper chelate by treatment with cupric acetate in ethanol and acetic acid. The remaining two components obtained after removal of 2-hydroxyalkanoic acid were fractionated by silica gel (BDH, 60-120 mesh) column chromatography to afford the individual components. An α,β-unsaturated acid, isolated by elution with petroleum ether-ether (95:5, V/V, yield 50%), was further purified by crystallization from petroleum ether-ether (75:25, V/V) m.p. 76°, lit.[112], mp 75.5 - 76°). The structure of α-β-unsaturated acid was established by elemental and spectral analyses of its methyl ester (XVII) prepared by refluxing the acid (5 g) with absolute methanol (75 ml) and catalytic amount of sulphuric acid as described earlier. The spectral and combustion data of α-β-unsaturated acid are given below:

Methyl docos-*trans*-2-enoate (XVII)

Anal. Calcd for $C_{23}H_{44}O_2$: C, 78.4; H, 12.57

Found: C, 78.31, H, 12.5

IR (CCl_4): 1730 (C=C-$COOCH_3$), 1640 (C=C) and 970 (*trans* olefins) cm^{-1}.

NMR (CCl_4): τ 3.1 d,d (J=15 and 5 Hz;, 1H, β to ester carbonyl),4.0 d (J = 15 Hz with a small long range coupling, *trans*-olefinic proton, 1H, α to ester carbonyl), 6.30 (s, 3H, $-\overset{O}{\overset{\|}{C}}-OCH_3$), 8.7 (br, s, chain - CH_2-) and 9.12 (distorted t, 3H, terminal -CH_3).

Nitrosobromination of methyl docos-*trans*-2-enoate (XVII)

To the solution of methyl docos-*trans*-2-enoate (XVII) (2.0 g) in 100 ml ethanol was added 2.0 g of *iso*-amyl nitrite and cooled to about 0^{o} in an ice-salt bath. 2.5 ml of HBr was added dropwise with constant stirring in 30 min. The reaction flask was kept in a refrigerator at $0\text{-}5^{o}$ for about a month. The reaction mixture was worked up as usual. The reaction product showed the presence of three components on analytical TLC. The major component corresponded to starting material. Column chromatographic separation of the products revealed that only about 10% of the compound (XVII) has reacted. The compounds (XVIII) and (XIX) were formed to the extent of 6 and 4% respectively. The structure of compounds (XVIII) and (XIX) were corroborated with the help of microanalysis, IR and NMR.

Elution with petroleum ether gave starting compound (XVII) and subsequent elution with petroleum ether-ether (90:5, v/v) gave compound (XIX).

Anal. Calcd. for $C_{23}H_{43}O_4N_2Br$: C, 56.21; H, 8.75; N, 5.70. Found: C, 56.3; H, 8.73, N, 8.77.

IR (neat): 1730 (ester-$\overset{O}{\overset{\|}{C}}$--), 1640 (C=N), 1550 and 1360 (NO_2), 540 (C-Br) cm^{-1}.

NMR (CCl_4): τ 5.98 (t, 1H, -C*H*Br-), 6.36 (s, 3H, -$\overset{O}{\overset{\|}{C}}$-OC$H_3$),

8.65 (br, s, shielded chain methylenes), and 9.1 (distorted t, 3H, terminal methyl).

Subsequent elution with petroleum ether-ether (90:10, V/V) gave compound (XVIII). Combustion and spectral data are as under.

Anal. Calcd. for $C_{23}H_{44}O_3NBr$: C, 59.74; H, 9.52; N, 3.03. Found: C, 59.7; H, 9.53; N, 3.01.

IR (neat) 3300 (OH), 1730 (ester-$\overset{O}{\overset{\|}{C}}$-), 1640 (C=N), 540 (C-Br) cm^{-1}.

NMR ($CDCl_3$): τ 2.76 (br, 1H, = N-O*H*), 6.16 (t, 1H, -C*H*Br-), 8.34 (s, 3H, -$\overset{O}{\overset{\|}{C}}$-OC*H*$_3$), 8.75 (br, s, chain methylene protons), and 9.12 (distorted t, 3H, terminal -C*H*$_3$).

Reaction of nitrosyl bromide with fatty 1,2-diol

Preparation of fatty 1,2-diol (XXI)

Pure 2-hydroxyhexadecanoic acid (XX) (4.0 g, 0.0156 mole) was esterified with absolute methanol (50 ml) containing catalytic amount of sulphuric acid by heating under reflux for 4 hr. The reaction product was extracted with ether, washed and dried over anhydrous sodium sulphate. Evaporation of the solvent gave methyl 2-hydroxyhexadecanoate as a white solid, mp 56-56.5°. The ester (3.8 g, 0.0132 mol) in dry ether (90 ml) was added to a well stirred

solution of lithium aluminium hydride (3.8 g) in dry ether (90 ml) at room temperature. The stirring was continued for 1 hr and the excess of reagent was decomposed by a mixture of cold ether-ethyl acetate (95:5, V/V) and cold 10% sulphuric acid. The product was extracted with ether. The combined ether extracts were washed with water and dried over anhydrous sodium sulphate. Evaporation of ether yielded a solid which on crystallization from ethanol gave pure 1,2-hexadecanediol (3.4 g, XXI), m.p. 74-75^{o} (lit.[113], m.p. 73.1-73.6^{o}).

Anal. Calcd. for $C_{16}H_{34}O_2$: C, 74.36; H, 13.26.

Found: C, 74.29, H, 13.28.

IR (KBr): 3440 br (OH), 2910, 1480, 1150, 1070, 990, 975, 880 and 730 (C-O) cm^{-1}.

NMR (CCl_4): τ 6.4 (1H, -C*H*(OH)-C*H*$_2$(OH)), 6.8 (d like, 2H, -CH(OH)-C*H*$_2$(OH)), 8.7 (br s, chain -C*H*$_2$-), and 9.15 (distorted t, 3H, terminal -C*H*$_3$).

Reaction of nitrosyl bromide with 1,2-diol (XXI)

The 1,2-hexadecanediol (XXI, 1.0 g, 0.0038 mole) was treated with NOBr *in situ* (*iso*-amyl alcohol + HBr) in ethanol (25 ml) for 6 hr at room temperature. After the usual work-up the product showed three distinct spots on TLC out of which one corresponded to the spot of starting

compound (XXI). The product (~ 0.88 g) was chromatographed over a column of silica gel (15 g) and the elution was carried out with petroleum ether containing increasing amount of ethyl ether. Elution with petroleum ether gave *iso*-amyl 2-nitritohexadecanoate (XXII, 0.21 g).

Anal. Calcd. for $C_{21}H_{41}O_4N$: C, 67.88; H, 11.12; N, 3.76. Found: C, 67.84; H, 11.10; N, 3.79

IR (neat) 1730 (ester $-\overset{\overset{O}{\|}}{C}-$), 1630 (-O-N=0) cm^{-1}.

NMR (CCl_4): τ 5.9 (mc, 3H, $-OCH_2-CHO$), 8.4 (1H, $-C\underline{H}<$), 8.7 (br s, shielded methylene protons), 9.1 (apparent d, methyl protons).

Mass: m/e 201 (1.7), 191 (0.8), 188 (0.6), 187 (2.3), 173 (0.4), 157 (4.5), 117 (3.8), 87 (3.3), 86 (3.9), 85 (9.0), 73 (2.1), 72 (6.5), 71 (100.0), 70 (12.0), 69 (9.5), 58 (1.8), 57 (11.4), 56 (5.8), 55 (18.7), 53 (1.7).

Subsequent elution with a mixture of petroleum ether-ether (95:5, V/V) gave *iso*-amyl 2-hydroxyhexadecanoate (XXIII, 0.32 g).

Anal. Calcd. for $C_{21}H_{42}O_3N$: C, 70.73; H, 11.87; N, 3.92. Found: C, 70.76; H, 11.88, N, 3.94.

IR (neat) : 3350 (OH), 1730 (ester $-\overset{\overset{O}{\|}}{C}-$) cm^{-1}.

NMR ($CDCl_3$): τ 5.9 (mc, 3H, -CH-OH and $-O-CH_2-$), 7.3 (1H, CHO*H*, D_2O exchangeable), 8.4 (1H, $-CH\langle^{CH_3}_{CH_3}$), 8.65 (br s,

shielded chain methylenes), 9.05 (apparent d, 9H, methyls). Mass: 327 (0.9), 313 (1.2), 295 (0.7), 294 (0.7), 286 (0.9), 284 (6.7), 226 (7.0), 174 (19.8), 133 (7.1), 132 (100.0), 116 (4.4), 113 (4.9), 111 (5.6), 109 (5.0), 104 (4.2), 100 (4.6), 99 (6.6), 97 (10.2), 95 (8.6), 90 (47.8), 85 (11.6), 84 (4.3), 83 (12.5), 82 (6.4), 81 (12.3), 74 (4.1), 71 (18.2), 70 (5.4), 69 (17.7), 68 (4.9), 67 (9.3), 57 (34.9), 56 (8.9), 55 (27.6), 54 (4.9).

Subsequent elution with petroleum ether-ether (80:20; V/V) gave starting material (XXI, 0.25 g) as a solid mp and mp 74.75.

Reaction of nitrosyl bromide with 10,11-epoxyundecanoic acid (XXV)

Preparation of 10-11-epoxyundecanoic acid[110]

The undecanoic acid (1.5 g) reacted with m-chloroperbenzoic acid (1.2 g) in chloroform (150 ml) at room temperature for 3-4 hr. The epoxy acid, recovered by ether extraction in almost quantitative yield, was purified by preparatgive TLC using silica (1 mm) and petroleum ether-ether (80:20, V/V) as developing solvent).

Reaction of nitrosyl bromide with 10-11-epoxy undecanoic acid (XXV)

The solution of 10-11-epoxyundecanoic acid (1 g) in ethanol (25 ml) was treated with NOBr *in situ* (*iso*-amyl

alcohol + HBr) with constant stirring at 0^{o} for $1\frac{1}{2}$ hr. After the usual work-up the compound showed a single spot on TLC having R_f value slightly lower than the epoxy compound showing thereby a quantitative yield of the product (XXVI, 0.9 g).

Anal. Calcd. for $C_{11}H_{20}O_4NBr$: C, 42.58; H, 6.45; N, 6.45. Found: C, 42.63; H, 6.44; N, 6.47.

IR (neat) 1770 (acid $-\overset{O}{\overset{\|}{C}}-$), 1630 (-ON=0), 550 (C-Br).

NMR (CCl_4): τ 2.47 (s, 1H, $-\overset{O}{\overset{\|}{C}}--OH$), 6.12 (1H, $-CHBr-$), 6.4 (d, 2H, $-O-CH_2-$), 7.7 (2H, protons α to -COOH group), and 8.65 (br s, shielded methylene protons).

Compound (XXVII) was obtained by the debromination of compound (XXVI) by using method of Jungermann and Spoerri[114] as described under.

To the solution of the compound (XXVI, 0.25 g) in 50 ml of glacial acetic acid, zink amalgam (1.0 g) was added. The mixture was refluxed for 6 hr. The reaction mixture was extracted with ether after dilution with water. The ethereal extract was dried over anhydrous sodium sulphate and evaporated to dryness. The product was not found pure as shown by analytical TLC. The NMR of impure sample, however, showed all the signals displayed by compound (XXVI) except the signal at τ 6.12.

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A. LIST OF PUBLICATIONS

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B. PAPER COMMUNICATED

(1) A Rich Source of Cyanolipid: ***Lepisanthes tetraphylla*** Seed Oil.